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**DETECTION, DISCHARGE AND ECOLOGICAL BEHAVIOUR
OF GENOTOXIC ORGANIC CONTAMINANTS IN THE ST.
LAWRENCE AND SAGUENAY RIVERS**

**Paul Andrew White
Department of Biology,
McGill University**

**A thesis submitted to the Faculty of Graduate Studies and Research in
partial fulfillment of the requirements for the degree Doctor of Philosophy**

November, 1995.

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ISBN 0-612-12510-6

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ABSTRACT

The goal of this thesis was to examine the sources and ecological behaviour of genotoxic organics in the St. Lawrence river system. A rapid and effective version of the SOS Chromotest was developed to accomplish the task of genotoxicity assessment. The method, validated with standard reference materials, is particularly well suited to complex environmental extracts. The endpoint investigated throughout the thesis, SOS genotoxicity, is empirically related to more familiar endpoints such as mutagenicity and carcinogenicity. Analyses of literature data confirmed correlations between SOS genotoxicity and both Ames/*Salmonella* mutagenicity and animal carcinogenicity. Analyses of industrial and municipal wastewaters revealed that organic and inorganic chemical production facilities, metal refining and founding facilities and petroleum refineries release high potency wastes. Loading values indicate that low potency sources (e.g. municipal wastewater and pulp & paper facilities) often have exceptionally high loadings. A substantial portion (2.3 - 99.8 %) of industrial genotoxins are associated with suspended particulates. Sorption partition coefficients ($K_d\text{-genotox}$) indicate that municipal wastewater and pulp & paper facilities release particularly soluble genotoxins. Extracts of suspended particulate matter collected near industrial discharges are direct-acting. Bottom sediment extracts are less potent and usually require S9 metabolic activation. Genotoxicity of both bottom sediment and suspended particulates are empirically related to the genotoxicity of regional industrial emissions. Direct-acting substances in the water column appear to be less stable and may be converted to more stable pro-genotoxic substances present in the bottom sediments. A mass balance of genotoxicity for St. Lawrence surface waters revealed that domestic, rather than industrial sources make the largest contribution to observed genotoxicity. The importance of domestic wastes was confirmed by extending the analyses to include the Great Lakes and several other rivers throughout the world. The SOS Chromotest was also used to detect genotoxic substances in aquatic biota. Tissues extracts of *Mya arenaria* from the Saguenay Fjord confirmed an accumulation of direct-acting substances. Likely sources include industrial and domestic wastes entering the Fjord via direct aqueous and airborne emissions. Investigations of genotoxins in fish and macroinvertebrates indicated that although genotoxins are accumulated, they are bio-diminished rather than biomagnified. Tissue to sediment ratios of genotoxicity indicate ecological behaviour that is similar to polycyclic aromatic hydrocarbons.

RÉSUMÉ

L'objectif de cette thèse était d'examiner les sources et le comportement écologique des substances organiques génotoxiques dans le Fleuve St-Laurent. Une version rapide et efficace du bioessai SOS Chromotest a été développée pour déterminer la génotoxicité de compartiments environnementaux. Cette méthode, qui a été validée avec des échantillons standards, est particulièrement bien adaptée aux extraits environnementaux complexes. Le critère d'effet examiné dans cette thèse, soit la génotoxicité du système SOS, est empiriquement relié à d'autres critères d'effet plus connus telles la mutagénicité et la cancérogénicité. Une analyse de la littérature a confirmé la corrélation entre la génotoxicité du système SOS, la mutagénicité du test d'Ames et la cancérogénicité animale. Les analyses des effluents industriels et municipaux ont démontré que les usines de fabrication de composés organiques et inorganiques, les industries métallurgiques ainsi que les raffineries pétrolières rejettent des déchets ayant une intensité toxique élevée. Les sources ayant une faible intensité toxique (e.g. effluent municipal et industrie de pâtes et papiers) avaient souvent des apports de substances toxiques remarquablement élevés. Une proportion importante (2,3-99,8%) des génotoxines d'origine industrielle était associée aux particules en suspension. Les coefficients d'adsorption ($K_d\text{-génotox}$) indiquent que les effluents municipaux et les industries de pâtes et papiers rejettent principalement des génotoxines solubles. Les génotoxines extraites des particules en suspension récoltées près des rejets industriels agissaient sans activation métabolique, alors que les génotoxines extraites des sédiments avaient une intensité toxiques plus faible et nécessitaient généralement une activation métabolique. La génotoxicité des sédiments et des particules en suspension était reliée empiriquement aux émissions industrielles régionales de génotoxines. Les substances à effets directs retrouvées dans la colonne d'eau semblaient moins stables et seraient probablement transformées en génotoxines plus stables à effets indirects qu'on retrouve dans les sédiments. Le bilan massique des génotoxines des eaux de surface du St-Laurent a indiqué que les effluents municipaux contribuaient davantage à la génotoxicité observée que les rejets industriels. L'importance des effluents municipaux concernant l'apport de génotoxines dans les écosystèmes aquatiques a été démontrée également avec les Grands Lacs et plusieurs autres rivières à travers le monde. Le bioessai SOS Chromotest a également été utilisé pour détecter les substances génotoxiques présentes dans les organismes aquatiques. Les extraits de tissus de *Mya arenaria* récolté dans le Fjord du Saguenay ont confirmé l'accumulation de génotoxines à effets directs. Ces substances proviennent probablement des effluents et des rejets atmosphériques industriels et municipaux. Nos résultats indiquent que les génotoxines étaient biodiminuées chez les

poissons et les macroinvertébrés plutôt que bioamplifiées dans la chaîne alimentaire. Le rapport de la génotoxicité des tissus sur celle des sédiments indique que le comportement écologique des génotoxines était similaire aux hydrocarbures aromatiques polycycliques (HAP).

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DEDICATION

I dedicate this thesis to the memory of a great man. A man who had a greater effect on my life than he ever knew. He taught me that a life of research can be a wondrous intellectual challenge on the road to discovery. He also taught me the meanings of the words community, philanthropy, compassion and generosity. I dedicate this thesis to Harold Nathan Segall, scientist, teacher, philanthropist and friend. I miss you Harold.

The significant problems we face cannot be solved at the same level of thinking we were at when we created them.

Albert Einstein

REMARKS ON STYLE AND AUTHORSHIP

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If this option is chosen, connecting texts that provide logical bridges between the different papers are mandatory. The thesis must be written in such a way that it is more than a mere collection of manuscripts; in other words, results of a series of papers must be integrated.

The thesis must still conform to all other requirements of the "Guidelines for Thesis Preparation". The thesis must include: A Table of Contents, an abstract in English and French, an introduction which clearly states the rationale of the study, a comprehensive review of the literature, a final conclusion and summary, and a thorough bibliography or reference list.

Additional material must be provided where appropriate (e.g. in appendices) and in sufficient detail to allow a clear and precise judgement to be made of the importance and originality of the research reported in the thesis.

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This thesis is divided into eight chapters. All of the chapters have been prepared for submission to peer-reviewed, scholarly journals. To date, chapters 1, 2, 3, 4 and 7 have

been submitted for peer-review. I designed and executed the entire study myself, in close co-operation with my supervisor Joseph Rasmussen. Professor Rasmussen played an active role in the development of the ideas presented in all eight chapters. As such, he is a co-author on all manuscripts. Dr. Christian Blaise of the St. Lawrence Center (Environment Canada) played an active role in the development of material presented in Chapters 1, 3, 4, 5 and 7. He appears as third author on the manuscripts developed from the material presented in Chapters 1, 3, 4 and 5. He is second author on the manuscript developed from the material in Chapter 7. His expertise and experience with microbioassays were invaluable in developing the final version of Chapter 1. His familiarity with the use of microbioassays for toxicity assessment resulted in useful contributions to Chapters 3, 4 and 5. His experience with *Mya arenaria* in the Saguenay Fjord were invaluable in the development of the material presented in Chapter 7. Chapter 1 has been accepted, with minor revisions, for publication in *Mutation Research*. Chapter 2 has been accepted, with minor revisions, for publication in *Environmental and Molecular Mutagenesis*. Chapters 3 and 4 are scheduled to be published as companion manuscripts in volume 26 of *Environmental and Molecular Mutagenesis*. Chapter 5 has been prepared for submission to *Environmental Toxicology and Chemistry*. Chapter 6 has been prepared for submission to *Nature*. Chapter 7 was submitted to *Mutation Research* in October 1995. Chapter 8 has been prepared for submission to *Environmental Toxicology and Chemistry*.

CONTRIBUTIONS TO ORIGINAL KNOWLEDGE

This thesis represents the first effort to use a non-target approach to examine the sources and ecological behaviour of genotoxic, organic substances discharged by a wide range of industrial and domestic sources. Rather than studying particularly interesting effluents, sediment or biota samples, the thesis examines a wide range of industrial discharges and extends these analyses well beyond the end of the pipe. The specific contributions include:

Chapter 1-

The testing of complex environmental extracts poses some methodological hurdles that must be overcome. In addition, environmental monitoring of large numbers of samples requires an assay system that is rapid, efficient and precise. This chapter outlines modifications to the existing SOS Chromotest protocols for the effective, rapid analysis of complex environmental extracts. The modifications presented are particularly well adapted to the testing of complex samples. The chapter also presents an unbiased method for the interpretation of SOS Chromotest results.

Chapter 2-

Although short-term microbial assays such as the SOS Chromotest are frequently used to assess genotoxicity, readers who are not familiar with SOS genotoxicity require a broader context within which they can interpret the results presented. This chapter illustrates, through in depth analyses of published data, empirical relationships between SOS genotoxic potency and both Ames/*Salmonella* mutagenic potency and animal carcinogenic potency. Although Philippe Quillardet (Institut Pasteur, Paris) and colleagues have investigated the qualitative correspondence between SOS Chromotest responses and both Ames/*Salmonella* assay responses and animal carcinogenicity assay responses, a thorough empirical analysis of potency data has never been presented.

Chapter 3-

This chapter presents the results of SOS Chromotest analyses of 42 industrial effluents belonging to eight industrial sectors. This is the first study to use daily discharge values to calculate total daily genotoxic loadings from a wide range of industries. The results confirm substantial differences in both the potency and the loading of wastes from different sectors. Few researchers have investigated a wide range of industrial discharges, and no previous studies have been able to identify significant differences between industrial

sectors. Fractionation of effluent extracts provided information about the physical-chemical properties of the putative genotoxins.

Chapter 4-

Although other researchers have investigated the tendency of organic contaminants (e.g. pesticides, PCBs) to associate with particulate matter, bioassays have never been used to investigate the partitioning behaviour of (geno)toxic complex mixtures. This chapter expands on the results presented in Chapter 3 and contains the first investigation of the partitioning behaviour of genotoxins in industrial discharges. The implications of particle adsorption for exposure of downstream biota are described and discussed.

Chapter 5-

As mentioned in the general introduction, sediment genotoxicity results are rarely used to investigate the persistence of genotoxins. This chapter provides the first discussion of the persistence of organic genotoxins in the aquatic environment. In addition, researchers rarely investigate potential industrial sources. Comparisons of sediment data with the industrial effluent loading data presented in Chapter 3 are used to provide insight into the post-emission behaviour of genotoxins discharged into the St. Lawrence and Saguenay rivers.

Chapter 6-

Traditional mass balance approaches used to quantify contaminant sources and fates are limited by their inability to integrate toxicity information. This chapter is the first mass balance analysis of complex toxic inputs. The mass balance is based on toxic loadings and does not require *a priori* knowledge of toxicant identity. The results indicate that domestic, rather than industrial wastes account for most of the observed surface water levels of genotoxicity.

Chapter 7-

For reasons discussed in Chapter 7, researchers interested in the accumulation of genotoxins in aquatic biota rarely use bioassays to investigate complex tissue extracts. This chapter is the first demonstration of the utility of the SOS Chromotest for the investigation of genotoxins in animal tissues. The results indicate that *Mya arenaria* in the Saguenay Fjord are accumulating genotoxic substances. Putative genotoxins appear to be entering the Fjord via airborne deposition and subsequent erosional transport, rather than direct industrial input.

Chapter 8-

Previous investigations of genotoxins in complex tissue extracts were primarily restricted to edible biota (e.g. shrimps, mussels, oysters). Fish are rarely investigated. In this chapter the SOS Chromotest method outlined in Chapter 1, and applied to biota analyses in Chapter 7, is used to investigate genotoxins in the tissues of aquatic biota collected at the sites investigated in Chapter 5. The results obtained are used to assess the tendency of genotoxins in aquatic systems to bioaccumulate and biomagnify.

ACKNOWLEDGMENTS

I am deeply indebted to many people for helping to make this thesis possible. First and foremost, I am grateful to my supervisor, Professor Joseph Rasmussen, for recognizing my potential and giving me the opportunity to be part of his research group. His unique insights and sometimes unconventional approaches were invaluable. His dedication, creativity and intellect are truly remarkable. I would also like to thank the other members of my supervisory committee, Drs. Jaap Kalff and Christian Blaise, for their continual support and encouragement. Dr. Blaise provided me with the opportunity to work at the St. Lawrence Center laboratories. In addition, it was Christian who first recognized the merits of the SOS Chromotest. Dr. Kalff always had his sights set on the bigger picture, and always looked out for my best interest. For that I am grateful.

I am indebted to many other academics, at McGill and elsewhere, who took the time to answer questions, discuss research problems and review draft manuscripts. These include Neil Price, Robert Peters and Pat Farrell of McGill University, Chris Metcalfe of Trent University, Douglas Bryant of McMaster University, Saburo Matsui of Kyoto University, John Meier of USEPA-Cincinnati, Virginia Houk of USEPA-Research Triangle Park, Kay Ho of USEPA-Naragansett, Kirby Donnelly of Texas A&M University, Stanislaw Pauwels of Exxon Biomedical, Emilien Pelltier of the Department of Fisheries and Oceans, Richard Carignan of the University of Montréal, Fernand Cadieux, Pierre Purenne and Michael Davidson of the Service de l'environnement, Communauté Urbaine de Montréal. I am also grateful to Philippe Quillardet of the Pasteur Institute, Audrey McDaniels of USEPA-Cincinnati and Paola Venier of the University of Padua for providing me with unpublished data.

There is no question that this project would not have been possible without the St. Lawrence Center. I am deeply indebted to the administration for allowing me to make use of their facilities. In particular I must thank Lynn Cleary, Raymond Vezeau and Normand Birmingham. In addition, I would like to thank the St. Lawrence Center personnel for their friendship and support. In particular I would like to thank Richard Legault, André Fouquet, Lucie Ménard, Sylvain Trotter, Manon Harwood, François Gagné, Daniel Boudreau, Thao Pham and Stephane Lorrain.

I am equally indebted to the graduate students and post-docs who enriched the time I spent at McGill. Most noteworthy are Gilbert Cabana, Paul del Giorgio, Marc Shallenberg, Lee Jackson, Jules Blais, Renate Lehmann, Sean Lonergan, Gavin Downing, Bettina Sanders, Josep Gasol, Marc Trudel, Helen Sarakinos, Jake Vander Zanden, Tony Ricciardi, Nell den Heyer and Roxanne Maranger.

Several summer research assistants assisted in the collection and processing of samples. I would like to thank Nell den Heyer, Jehangir Appoo, Brigid Payne, Jay Leopkey and Brian Walker.

For help with the Biomek™ 1000 automated laboratory workstation I must thank the Bioanalytical Systems Group of Beckman Canada Inc., Mississauga, Ontario. They graciously provided the labware required to develop the protocol presented in Chapter 1.

Finally, I must thank my family for their love and support. My parents, my sister and my brothers were always a source of encouragement. My parents always supported my work and took pride in my scholastic accomplishments. My dog Sofi was a never ending source of happiness. No matter how late I worked, no matter how little time I spent at home, she was always happy to see me and always wagged her tail. Last, but certainly not least, I must thank Chantale Côté. It is difficult to find the words required to express my gratitude for her support, encouragement and love. In addition to moral support, she frequently took the time and effort to review and comment on my work. Our frequent scientific discussions were invaluable and helped me to work through many research problems. She is a truly remarkable woman. I love her very much.

Financial support for this project came from a variety of sources. Research expenses were covered primarily by an Natural Sciences and Engineering Research Council of Canada-St. Lawrence Center research partnership grant to J. Rasmussen, a Wildlife Toxicology Fund grant (World Wildlife Fund) to J. Rasmussen, and an FCAR (Fondation Canadienne d'Aide à la Recherche) team grant to J. Kalff, J. Rasmussen and R. Peters. My own personal expenses were covered primarily by graduate student fellowships from FCAR, NSERC and McGill University (Vineberg Family Award).

GENERAL INTRODUCTION

Acute vs. Chronic Toxicity

Much of the research efforts in aquatic toxicology is concerned with acute toxicity (Giesy and Graney, 1989). Acute toxicity is readily detected by its rapid effects on exposed biota. Exposure levels are usually high and effects are generally severe (e.g. death). However, acute effects only represent the first level of impact of contaminants on aquatic ecosystems (van Coillie et al., 1989; de Raat et al., 1985). Some substances may not invoke any detectable acute response, but may reduce the long-term survival of exposed organisms through chronic effects. Such chronic effects are often manifested as excessive tissue damage, accumulation of persistent contaminants, or disorders that require long latency periods before becoming clinically visible (e.g. cancer). Some chronic toxicants exert their effects via irreversible damage to the genetic material of somatic and/or germ cells. These toxins are referred to as genotoxins. Genotoxins that can cause permanent changes in heritable genetic information are referred to as mutagens.

Genotoxic and mutagenic effects are varied and often severe (Kurelec, 1993). The most familiar genotoxic effects are gene pool alteration via germ cell mutation, and neoplastic transformation through somatic cell mutation. Although the former effect is perhaps the most alarming, it receives much less attention than the latter. Research aimed at investigating the relationship between genotoxicity and carcinogenicity has preoccupied an enormous number of researchers and promoted the development of genetic toxicology as a discipline. Although clear mechanistic (see review by Burck et al., 1988) and empirical (e.g. Meselson and Russel, 1977; Ashby and Tennant, 1988; McCann et al., 1988; Kier, 1988; Parodi et al., 1991) links between mutagenicity and carcinogenicity do exist, the role of mutagenesis in tumour initiation is extremely controversial (e.g. Lijinsky, 1989; Ames and Gold, 1990).

Genetic Toxicology - An Historical Perspective

The roots of genetic toxicology can be found in a number of related disciplines such as occupational health and safety, oncology, radiation medicine and genetics. Interest in environmental carcinogens can be traced to the 18th century works of Percival Pott, a British surgeon who attributed the high frequency of scrotal cancer (*soot-wart*) in chimney sweeps to soot exposure (Pott, 1775; Earle, 1823). Further investigations carried out in the late 19th and early 20th century confirmed the carcinogenic hazards of chimney soot, coal tar, and various coal tar and shale oil distillates (Bell, 1876; Butlin, 1892a; Butlin, 1892b; Passey, 1922; Kennaway, 1925). Parallel research efforts aimed at investigating gene

mutability, documented the ability of both physical (e.g. X-radiation, Muller, 1927) and chemical (e.g. sulfur mustard, Auerbach and Robson, 1946) agents to cause mutations. By the 1930's, British researchers (Kennaway, 1925; Kennaway, 1955) had successfully isolated several carcinogenic polycyclic aromatic hydrocarbons (PAHs) from coal tar. Subsequent investigations in the 1940's and 1950's were aimed at determining the relationship between the carcinogenicity of these substances and their ability to induce somatic cell mutations. Such studies were attempts to validate the *somatic mutation theory of cancer*, put forward by Theodor Boveri some 30 years earlier. As early as 1914 Boveri reasoned that ".....if we have to consider a malignant cell as a cell with an irreparable defect, this defect is located, not in the protoplasm but in the nucleus" (Boveri, 1929, p. 37).

By the 1960's it was becoming increasingly clear that some carcinogens are not genotoxic unless they are enzymatically transformed *in vivo* into electrophilic substances that are capable of reacting with DNA (e.g. Miller and Miller, 1961; Miller and Miller, 1966). Subsequent research (Malling, 1971) demonstrated that the required *in vivo* metabolic activation could be simulated *in vitro*. The *in vitro* system confirmed that well known carcinogens such as dimethylnitrosamine are indeed mutagenic to prokaryotes such as *Salmonella typhimurium* (Malling, 1971). By 1975, Ames and colleagues (e.g. Ames et al., 1973; McCann et al., 1975) demonstrated that large numbers of known carcinogens are mutagenic. Despite conflicting evidence that some carcinogens are not mutagenic (e.g. diethyl stilbestrol, carbon tetrachloride; see Weisenburger and Williams, 1982 for review), a flurry of subsequent research efforts were aimed at detecting mutagenic substances that could constitute a serious human health hazard.

Increased support for the somatic mutation theory of cancer initiated the development of a wide range of bioassays for mutagen detection (Li and Loretz, 1991). The most popular tests are the short-term microbial assays such as the *Salmonella* /Mammalian Microsome Assay developed by Ames and colleagues (Ames et al., 1973; Ames et al., 1975b). This test and other short-term mutagenicity and genotoxicity assays such as *Saccharomyces spp.* reverse mutation assays (Davies et al., 1975), *Escherichia coli* reverse mutation assays (Brusick et al., 1980), the *Escherichia coli umu* test (Oda et al., 1985), the SOS Chromotest (Quillardet et al., 1982), and the Microscreen phage induction assay (Rossman et al., 1984) dramatically reduce the time and effort required for assessment of genotoxicity and mutagenicity.

Complex Mixtures. A Non-Target Approach to Aquatic Toxicology.

The conventional, chemical-specific approach to aquatic toxicology was developed in

the 1960's and 70's when few pollutants were known to accumulate in the environment. As such, the approach is ill-equipped to handle the enormous number of chemicals presently being released into the environment (DOE, 1989). Attempts to focus the chemical-specific approach have produced lists of substances that are *priorities* for concern and control (DOE, 1989). While priority lists do provide focus, they encourage the allocation of large amounts of analytical effort for the detection and control of a small number of substances, with little or no effort allocated towards uncharacterized, *non-priority* substances.

Over the past 25 years the Ames/*Salmonella* assay and other short-term, microbial genotoxicity and mutagenicity assays have been successfully used to identify specific mutagenic substances such as dyes and toners (e.g. Ames et al., 1975b; Rosenkranz et al., 1980), pesticides (e.g. Shirasu et al., 1976; Simon et al., 1977), food additives (e.g. Joner et al., 1977; Sugimura and Sato, 1983) and drugs (e.g. Dayan et al., 1982; Rosenkranz and Speck, 1975). Appropriate measures were subsequently instituted to ensure containment and control. Despite these successes, chemical-specific analyses ignore the fact that organisms outside the laboratory are exposed to complex mixtures of unknown substances. Genetic toxicologists were among the first toxicologists to recognize this fact and employ bioassays to investigate complex environmental samples such as urban air particulates, cigarette smoke condensate and automobile exhaust (e.g. Chrisp and Fisher, 1980; Kier et al., 1974; Wang et al., 1978).

Most investigations of complex mixtures are oriented towards the ultimate identification of putative genotoxins or mutagens. In many instances, identification of putative genotoxins is attempted via simultaneous detailed chemical analyses. However, several studies have indicated that the (geno)toxicity of complex samples can rarely be accounted for by the concentration of identified toxicants (e.g. Ellis et al., 1982; Jolley et al., 1982; Hendriks et al. 1994; Grifoll et al., 1990). In addition, detailed chemical analysis is limited in its ability to predict the toxicity of complex mixtures (Brouwer et al., 1990; Samoiloff, 1983; Houk, 1992). An alternative approach involves augmenting bioassay procedures with chemical fractionation techniques. Observed (geno)toxic responses to complex samples can be traced through a serial fractionation protocol in an effort to ultimately identify putative (geno)toxicants (Schuetzle and Lewtas, 1986; Alfheim et al., 1984). Such *bioassay-directed fractionation techniques* have been successfully used to investigate mutagens in urban air particulate matter (e.g. Sicherer-Roetman et al. 1988; Nishioka et al., 1988), diesel particulate matter (e.g. Saleem et al., 1984; Pedersen, 1983), contaminated sediments (e.g. Ho and Quinn, 1993; Marvin et al., 1993), drinking water (e.g. Horth et al., 1987; Kronberg et al., 1988) and industrial effluents (e.g. Nestmann et

al., 1979; Holmbom et al., 1984).

Genotoxins in the Aquatic Environment

Early observations of mutagenic chemicals, primarily PAHs, in surface waters (e.g. Harrison et al., 1974; Andelman and Suess, 1970), sediments (e.g. Johnson et al., 1985; Black, 1983; Stich and Dunn, 1980) and aquatic biota (e.g. Zitko, 1975; Eisler, 1987), as well as early observations of neoplastic diseases in fish from polluted regions (e.g. Brown et al., 1973; Sonstegard, 1977), led to increased concern about the presence, origins and effects of genotoxins in the aquatic environment. Evidence that identified mutagens may only represent a minor fraction of the total mutagens present in surface waters led to the increased use of bioassays to investigate complex mixtures of genotoxins, mutagens and carcinogens in the aquatic environment (e.g. van Kreijl and Sloof, 1985; Pelon et al., 1977; Metcalfe et al., 1990). Parallel investigations of industrial effluents, documented the genotoxicity of aqueous wastes that are directly discharged into surface waters (see Houk, 1992 for review).

Well over 100 studies have used bioassays to investigate genotoxins in surface waters, sediments and industrial effluents (see reviews by Stahl, 1991 and Houk, 1992). The premise employed in the vast majority of these studies is the isolation and identification of putative genotoxins that presumably constitute a serious health hazard to exposed humans and indigenous biota. This goal is usually accomplished through intensive study of a single sample or a small number of samples. While intensive efforts aimed at the isolation and identification of genotoxins in industrial effluents, surface waters and sediments have been successful (e.g. Ho and Quinn, 1993; Balch et al., 1995; Maruoka et al., 1986; Holmbom et al., 1984), such studies are limited in scope. While the techniques employed are often innovative and progressive, the results obtained are difficult to evaluate in a broader context. The ultimate hazard of the identified substances will be determined by loading, bioavailability, persistence and potency.

Genetic Ecotoxicology

Ecotoxicology is the study of the fate and effects of contaminants on ecosystems (Shane, 1994). As such, it approaches the problem of contamination of aquatic habitats from a very broad perspective. The tendency of genetic toxicology to focus on sample potency and the chemistry of particularly potent samples is a noteworthy shortcoming. While it provides mechanistic insights into mutagenesis and the chemistry of genotoxin production, it fails to consider the (aquatic) ecosystem "as a whole" (Moutschen, 1985, p. 115). For example, very few investigations of industrial discharges attempt to calculate

daily loading rates, the value required to assess potential downstream hazard. Furthermore, despite numerous examinations of water, sediment and biota, researchers rarely investigate, or comment on, ecotoxicological phenomena such as bioaccumulation and persistence.

The most hazardous genotoxic contaminants are those that tend to accumulate in the tissues of exposed biota and are resistant to degradation. Previous research efforts in aquatic toxicology have identified several physical-chemical attributes of organic contaminants that increase environmental hazard. The most important is water solubility, often expressed in terms of the octanol-water partition coefficient (P_{oct} or K_{ow}). Compounds with high K_{ow} 's are highly lipophilic, have a tendency to accumulate in aquatic biota (Chiou et al., 1977; Mackay, 1982; Spacie and Hamelink, 1985), and a tendency to associate with particulate material (Karickhoff et al., 1979; Schwarzenbach and Westall, 1981; Means et al., 1980). The ability of high K_{ow} substances to cross biological membranes also contributes to increased genotoxic and mutagenic potency (Lopez de Compadre et al., 1988; Debnath et al., 1992; Debnath and Hansch, 1992).

Recent research confirmed that food chain length is also an important determinant of the levels of persistent organic contaminants in aquatic biota (Oliver and Niimi, 1988; Rasmussen et al., 1990). Thus, the accumulation of genotoxins in aquatic biota will likely be dependent on K_{ow} as well the ability of exposed biota to assimilate, metabolize and excrete such substances. While fish are known to be capable of metabolizing and excreting some genotoxic substances (Stegman and Lech, 1985), the ability of aquatic invertebrates to metabolize and excrete genotoxins is often limited and generally less well understood (e.g. Lee, 1981; Livingstone, 1994; James, 1989). Resistance to degradation can also result in accumulation of genotoxins in sedimented particulate material with subsequent sediment resuspension and redistribution (Karickhoff and Morris, 1985; Elder et al., 1979) resulting in continual exposure. Although the half-life of genotoxins in the aquatic environment has not been explicitly addressed, published information suggests that some substances are likely to be rapidly degraded, while others may persist for long periods of time. Debnath and Hansch (1992) demonstrated that molecular orbital energy levels can determine the ease with which a mutagen undergoes reduction to a reactive species. Highly reactive species might be expected to be readily degraded, or readily transformed into more stable substances. Conversely, the accumulation of genotoxic PAHs in sediments indicates that they are relatively persistent (Balch et al., 1995; Marvin et al., 1994; Ho and Quinn, 1993).

This thesis is an attempt to investigate the sources, movements and ecological behaviour of organic genotoxins discharged into several Québec rivers. The thesis is essentially divided into three sections. The first section (Chapters 1 and 2) describes a

semi-automated version of the SOS Chromotest for the detection of genotoxins in complex environmental samples and, through analyses of literature data, presents a broad empirical framework within which SOS Chromotest results can be interpreted. The second section (Chapters 3 and 4) uses the SOS Chromotest procedure outlined in Chapter 1 to investigate the presence, potency, potential hazard and partitioning behaviour of organic genotoxins in a wide range of industrial effluents. The third and final section of the thesis (Chapters 5 through 8) investigates the post-emission, ecological behaviour of organic genotoxins discharged into the St. Lawrence and Saguenay rivers. In Chapter 5, examinations of suspended and sedimented particulate matter are used to investigate the fate of genotoxins in surface waters. In Chapter 6 a mass balance approach is used to identify the industrial and municipal sources that are responsible for the observed surface waters levels of genotoxicity. In Chapters 7 and 8, examinations of macroinvertebrates and fish are used to investigate the bioaccumulation and biomagnification of genotoxins in aquatic habitats.

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CHAPTER 1

A SEMI-AUTOMATED, MICROPLATE VERSION OF THE SOS CHROMOTEST FOR THE ANALYSIS OF COMPLEX ENVIRONMENTAL EXTRACTS

INTRODUCTORY REMARKS

University regulations require the inclusion of connecting texts that provide logical bridges between chapters that include the text of manuscripts submitted for publication. As such, each chapter includes a short introductory comment.

When the research project was conceived, it was apparent that an effective tool for genotoxicity assessment is required. The SOS Chromotest seemed to be a promising short-term, microbial assay for the rapid analysis of complex environmental extracts. However, there were several problems that needed to be solved. Those problems and their solutions are discussed in this chapter.

The material presented in this chapter has been accepted, with minor revisions, for publication in *Mutation Research*. As outlined in the preface, both Prof. Joseph Rasmussen and Dr. Christian Blaise made significant contributions. As such, they are second and third authors respectively.

Appendices 1, 2 and 3 contain material related to this chapter. Appendix 1 contains the program used to control the Biomek™ 1000 automated laboratory workstation. Appendix 2 contains the program used to control the Multiskan™ microplate spectrophotometer. Appendix 3 contains the data used to generate all figures.

ABSTRACT

Environmental monitoring for genotoxicity requires that a large number of measurements be made across space and time. This requirement demands a rapid and efficient bioassay system. The SOS Chromotest is a rapid, efficient bacterial system for the detection of DNA damaging agents. Over 100 publications have described its use on a variety of samples. Relatively few studies have used the test to examine complex mixtures. Effective testing of complex samples poses a variety of problems. Although solutions have been proposed, few have validated the resulting protocol. In this work we present a semi-automated microplate version of the SOS Chromotest for the examination of complex mixtures. Experiments were conducted to determine the optimal cell concentration, exposure time, substrate conversion time and S9 enzyme concentration. The performance of the method was evaluated using 6 reference genotoxins and 3 complex mixtures. The complex mixtures examined are extracts of diesel particulate matter, urban dust/organics and coal tar. The results obtained indicate that optimal responses often require fewer cells ($\approx 5-10 \times 10^6$ CFU per mL) and a longer exposure (3 hrs) than that recommended in the original protocol. Interfering effects of colored and turbid samples are removed using centrifugation and initial optical density readings taken 60 min after cell resuspension and lysis. The performance of the established protocol was evaluated using mitomycin C and benzo(a)pyrene results for 207 microplates and solvent control results for 293 microplates. The results indicate that the established method is accurate, sensitive and precise. Coefficient of variation on mean SOSIP values for mitomycin C and benzo(a)pyrene are < 5%. Solvent control data indicate that the standard threshold for determination of a positive response (induction factor > 1.5) is excessively conservative. All liquid transfers were automated using the Biomek™ automated laboratory workstation. Automation permits a throughput of up to 72 samples per day and maintains excellent precision and accuracy.

INTRODUCTION

Genotoxic and mutagenic substances have been detected in a wide range of environmental samples including urban air particulates (reviewed by Chrisp and Fischer, 1980), gasoline and diesel engine emissions (e.g. Nishioka et al., 1983; Saleem et al., 1984; Wang et al., 1978); river sediments (e.g. Langevin et al., 1992; Metcalfe et al., 1990; Durant et al., 1992), contaminated soils (e.g. Donnelly et al., 1983; Brown and Donnelly, 1988), surface waters (reviewed by Stahl, 1991), snow (White et al., 1995a) and industrial wastes (reviewed by Houk, 1992). A thorough understanding of the sources, pathways, sinks and potential hazard of these substances for both humans and other biota requires extensive environmental monitoring of the relevant environmental media. To be effective, environmental monitoring research requires that a large number of measurements be made over time and across locations (Lewtas, 1991). In addition, bioassay directed fractionation for the identification of genotoxins in complex samples requires that large numbers of samples be examined (e.g. Marvin et al., 1993; Meier et al., 1987; Savard et al., 1992; Schuetzle and Lewtas, 1986). Therefore, research investigating the genotoxicity of complex mixtures requires inexpensive, rapid and sensitive (micro)bioassays (Brusick, 1987; Blaise et al., 1988). The bioassay system that is most frequently used for the detection of genotoxic substances in complex environmental samples is the *Salmonella*/mammalian microsome assay (Ames et al., 1973; Ames et al., 1975, Maron and Ames, 1983). The *Salmonella* test detects mutagenic substances via their ability to revert histidine auxotrophs of *Salmonella typhimurium* to wild type. The test system was recently used in an extensive inter-laboratory, collaborative study on the mutagenicity of complex mixtures (Claxton et al., 1992). Since the advent of the *Salmonella* test many other short-term bacterial assays have been developed. Several assays (e.g. the Microscreen phage induction assay of Rossman et al., 1984; the SOS/*umu* test of Oda et al., 1985; the SOS Chromotest of Quillardet et al., 1982) use the error-prone DNA repair pathway of *Escherichia coli* for the detection of DNA damaging agents. The error-prone repair pathway, also known as the SOS response, is a complex regulatory network that is induced by DNA damage (Walker, 1984). Activation of the SOS system results in the coordinated regulation of a series (>17) of unlinked, *damage-inducible* genes that have a variety of known and unknown functions (Kenyon and Walker, 1980). Using the specialized transducing phage Mu *dl*(Ap, *lac*) (Casadaban and Cohen, 1979), Huisman and D'Ari (1981) constructed a strain of *Escherichia coli* in which the expression of β -galactosidase is under the express regulatory control of the SOS response pathway. This strain, known as PQ37, became the cornerstone of the SOS Chromotest for the detection of

DNA damaging agents. The test involves incubation of the bacteria with the substance under investigation and subsequent spectrophotometric determination of β -galactosidase activity - i.e. the level of SOS induction. Activity values are usually normalized to account for the background level of SOS induction in cells that are exposed to a solvent control. Constitutive synthesis of alkaline phosphatase, an enzyme not under SOS control, permits an indirect measure of bacteriostatic effects. The test system has many advantages that make it an attractive choice for environmental monitoring- 1) survival of the tester strain is not required; 2) the results can be obtained in a single working day; 3) sample sterility is not usually required; 4) the organism responds to a wide range of DNA damage scenarios (Elespuru, 1987; Houk and DeMarini, 1988); 5) bacteriostatic effects can be simultaneously monitored, 6) the microplate version of the assay is readily amenable to automation and 7) the test can easily accept biological samples such as tissue extracts and biological fluids (histidine contamination of biological samples can effect *Salmonella* test performance (Sparks et al., 1981; Parry, 1985; Fish et al., 1987; Kohn et al., 1988; De M  o et al., 1988; Bosworth and Venitt, 1986)). Since its inception in 1982 the test has been validated (Von der Hude et al., 1988; Quillardet et al., 1985; Ohta et al., 1984; Quillardet and Hofnung, 1993) and used to measure the genotoxicity of over 750 chemicals. However, despite over 100 publications, relatively few studies have used the assay to detect genotoxicity in complex environmental extracts. The test has recently undergone a number of modifications that optimize performance and efficiency. Microsuspension methods permit the entire assay to be carried out in microtiter plates. This facilitates sample processing and spectrophotometric measurements. Fish et al. (1987) described a kit version of the microtiter SOS Chromotest. Although convenient and efficient, the cost of the kit system (Environmental Biodetection Products Inc., Brampton, Ontario) often prohibits extensive environmental monitoring.

In this work we present a semi-automated, microsuspension SOS Chromotest for the detection of genotoxic responses in complex mixtures. The method decreases the time and expense of processing large numbers of samples. Three standard reference materials and six reference genotoxins were used to assess assay performance. The Biomek™ 1000 automated laboratory workstation (Beckman Instruments, Palo Alto, CA) performed all liquid transfers and serial dilutions required in the assay.

MATERIALS AND METHODS

Culture media, reagents, reference materials and laboratory equipment

LB (Luria-Bertani) medium: 10g Bacto tryptone (Difco Laboratories, Detroit, MI), 5g Bacto yeast extract (Difco), 10g NaCl (Fischer Scientific, Fair Lawn, NJ) and 20 mg ampicillin (Sigma Chemical Co., St. Louis, MO) per liter of Super Q™ (Millipore Corp., Bedford, MA) water.

Enzyme assay/cell lysis reagent or *SOS Chromogen*: 40% v/v methanol (spectrophotometric grade- Fischer), 0.5% v/v toluene (spectrophotometric grade- Fischer), 10% v/v N,N-dimethyl formamide (spectrophotometric grade- Fischer), 4 mg/mL 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (Vector Biosystems, Toronto, Ontario), 1 mg/mL *p*-nitrophenyl phosphate (Sigma). The reagent is prepared in Super Q™ water adjusted to a pH of 9 with 0.1 N NaOH. Immediately prior to use the reagent is warmed to 37°C and filtered (0.2 μ m cellulose-nitrate filter, Nalgene Labware, Rochester, NY). The final pH of the reagent is 7.8 to 7.9.

S9 Activation Mixture: 33 mM KCl (Sigma) 8 mM MgCl₂ (J.T. Baker Chemical Co., Phillipsburg, NJ), 100 mM (Tris[hydroxymethyl])-aminomethane pH 7.4 (Sigma), 5 mM glucose-6-phosphate (sodium salt, Boehringer-Mannheim Canada Ltd., Laval, Québec), 2 mM NADP (nicotinamide adenine dinucleotide phosphate, disodium salt- Boehringer-Mannheim) and 20-160 μ L of Arochlor 1254 induced rat liver S9 (Molecular Toxicology Inc., Annapolis, MD) per mL of activation mixture. Activation mixture was kept on ice until the start of the assay. S9 concentration values provided in the text reflect the concentration of S9 (in % v/v) in the final assay mixture (i.e. not in the S9 activation mixture).

Resuspension buffer: 200 mM (Tris[hydroxymethyl])-aminomethane pH 7.5 (Sigma).

Reference genotoxins: Mitomycin C (CAS #50-07-7, Sigma), 4-Nitroquinoline-1-oxide (CAS # 56-57-5, Aldrich Chemical Co., Milwaukee, WI), N-Methyl-N'-nitro-N-nitrosoguanidine (CAS # 70-25-7, Sigma), Captan (3a,4,7,7a-Tetrahydro-2-[(trichloromethyl)thio]-1H-isoindole-1,3(2H)-dione, CAS # 133-06-2, Chevron Chemical Co., San Francisco, CA), N-Nitrosodimethylamine (CAS # 62-75-9, Sigma), 2-Naphthylamine (CAS # 91-59-8, Sigma), Benzo(a)pyrene (CAS # 50-32-8, Sigma).

Standard Reference Materials: All standard reference materials (SRMs) were obtained from the U.S. National Institute of Standards and Technology, Gaithersburg, MD. The three SRMs used in this study are: SRM 1597- complex mixture of polycyclic aromatic hydrocarbons from coal tar, SRM 1649- urban dust/organics and SRM 1650- diesel particulate matter. SRM 1597 is a toluene extract of medium-crude coke oven tar. SRM

1649 is intended to typify atmospheric particulate matter obtained from an urban area. The material is a time-integrated sample collected over a twelve month period in the Washington, DC area. The sample was screened through a fine sieve to remove extraneous matter. SRM 1650 is intended to be a representative of heavy-duty diesel engine particulate emissions. The material was collected from several four-cycle diesel engines, operating under a variety of conditions.

Spectrophotometers- Titertek Multiskan MCC/340 microplate spectrophotometer (Flow Laboratories, Lugano, Switzerland) for microtiter plates, Beckman DU-70 UV-VIS spectrophotometer (Beckman Instruments) with sipper flowcell for monitoring bacterial cultures.

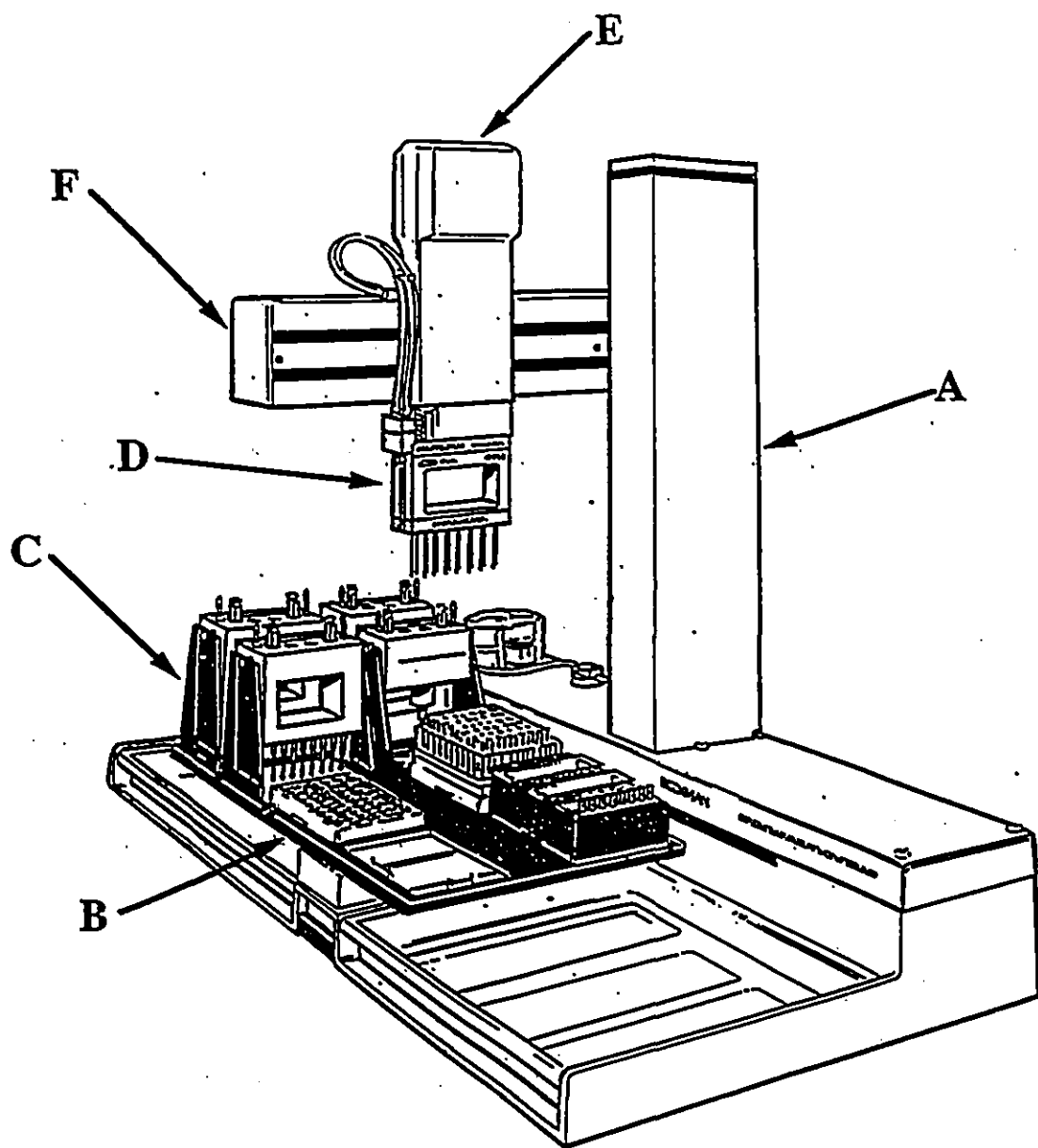
Microplate centrifuge- Heraeus Varifuge RF (Heraeus Sepatech GmbH, Am Kalkberg, Germany) with microplate rotor (model # 4690).

Automated laboratory workstation- Biomek™ 1000 (Beckman Instruments), controlled by the Genesis™ (version 2.11) software running on an IBM PS/2™ model 50 computer (IBM Corp., Armonk, NY). The instrument (without computer) is illustrated in Figure 1. Both multichannel and single channel pipette tools were used to manipulate volumes from 5 µL to 200 µL. When properly adjusted, the instrument can routinely deliver volumes from 2 µL and 1000 µL with less than 1% error (usually in the 0.2% to 0.6% range). The Genesis™ software contains a user-friendly interface that permits custom programming of the instrument. Custom subroutines permit liquid transfers, liquid mixing and serial dilutions. To provide optimal performance, liquid transfer parameters such as dispense rate, tip height, mixing speed and height, tip prewetting, liquid blowout or *to contain* volume, liquid level sensing and tip touching can be adjusted to suit the physical properties of the reagents and the desired degree of accuracy and precision. A custom program for the SOS Chromotest was written in our laboratory and is available on diskette from the corresponding author (see Appendix 1). Sterile polypropylene pipette tips (TiterPak™) were obtained from Robbins Scientific Corp., Sunnyvale, CA.

Tester strain maintenance and culture methods

Escherichia coli strain PQ37 (*F⁻ thr leu his-4 pyrD thi galE galK or galT lacΔU169 srl300::Tn10 rpoB rpsL uvrA rfa trp::Muc⁺ sulA::Mud(Ap,lac) cts PhoC*) was kindly provided by Philippe Quillardet (Unité de Programmation Moléculaire et Toxicologie Génétique, Institut Pasteur, Paris). Frozen permanent copies of the tester strain were prepared according to Maron and Ames (1983). Copies of the tester strain were stored at -80°C. The integrity of several genetic markers was verified semi-annually. The deep rough or *rfa* mutation was checked via the inability of the strain to grow on MacConkey agar

Figure 1. The Biomek™ 1000 Automated Laboratory Workstation. The instrument performed all liquid transfers and serial dilutions required for the SOS Chromotest. The instrument is made up of a series of moving parts that collectively permit three-dimensional movement. The elevator (A) provides vertical movement for the bridge (F) and pod (E). The bridge and the attached pod move up and down over the tablet (B). The pod moves back to front along the bridge and across the width of the tablet. The tablet moves side to side beneath the pod and bridge. The tablet contains four locations that hold currently used labware and four locations for storing pipetting tools currently not in use (C). The tool currently in use (D) is attached to the pod and carries out automated liquid handling and measurement functions. Compatible labware include a variety of microtiter plates and tube racks, a variety of pipette tips for volumes from 2-1000 µL and a wide range of reagent reservoirs. The instrument can be equipped with an optional sideloader that automatically removes used labware from the tablet and replaces them with a fresh supply as required.



**The Biomek™ 1000 Automated
Laboratory Workstation**

(Difco). Sensitivity to ultraviolet radiation, the *uvrA* mutation, was tested as described by Ames et al. (1975). Constitutivity for alkaline phosphatase (PhoC) was tested as described by Quillardet and Hofnung (1985). The integrity of the *sulA::lacZ* fusion was verified using a modified version of the standard SOS spot test (Mamber et al., 1986).

To prepare a PQ37 culture, a 1 mL frozen permanent copy was thawed and diluted in 50 mL of fresh LB broth (20 µg/mL ampicillin) in a 125 mL Erlenmeyer flask fitted with a cotton/cheesecloth plug. Flasks were incubated overnight (15 hr maximum) with agitation (200 rpm) at 37°C.

Extraction of SRMs (Standard Reference Materials)

SRM 1597 was supplied as a liquid in 5 mL sealed ampoules (1.3 mL toluene/ampoule). The "Certificate of Analysis" indicates that each µL of sample is equivalent to approximately 8 µg of coal tar. SRM 1650 and SRM 1649 were both supplied as a dry powder. Dichloromethane extracts of SRM 1650 and SRM 1649 were prepared using a combined blender/sonication method described by White et al. (1995b). The method is a variation on those developed by Marble and Delfino (1988), Maggard et al. (1987) and Williams (1989). Maggard et al. (1987) determined that, for mutagenicity studies, the performance of blender methods is similar to, or better than, that obtained using Soxhlet extraction. Nielsen (1992) determined that dichloromethane is the best choice as a general solvent for extraction of complex environmental samples. Briefly, each sample (2.0 g of SRM 1649 and 0.25 g of SRM 1650) was blended with 250 mL of dichloromethane in stainless steel cups using a high-speed (3500 rpm) industrial blender (Eberbach Corp., Ann Arbor, MI). Each sample was blended for 3 x 4 minutes and subsequently sonicated (ultrasonic cell disrupter- Branson Sonic Power Co., Danbury, CT) on ice for 2 x 3 minutes. Blending and sonication periods were alternated with 3-4 minute cooling periods. Extracts were dried with anhydrous sodium sulfate (Mallinckrodt Specialty Chemicals, Mississauga, Ontario), filtered through coarse sintered glass and reduced to approximately 5 mL by rotary evaporation at 30°C. Where necessary, fine particulate matter was removed via filtration through 0.45 µm Teflon™ filters (Gelman Science, Ann Arbor, MI). Extracts were taken to dryness under vacuum at low temperature (Speedvac AS290 Concentrator, Savant Ltd., Farmingdale, NY). Both extracts were resuspended in high purity dimethyl sulfoxide (DMSO) (Sigma). Samples of SRM 1597 were taken to dryness and resuspended in DMSO. Extracts were resuspended at the following concentrations: 250 mg of equivalent starting material per mL of DMSO for SRM 1650, 2.0 g of equivalent starting material per mL for SRM 1649 and approximately 8 mg of coal tar equivalent per mL for SRM 1597. Maximum tested concentrations were 100 mg

per assay mL for SRM 1649, 12.5 mg per assay mL for SRM 1650 and approximately 0.4 mg of coal tar equivalent (50 μ L of SRM) per assay mL for SRM 1597.

Initiation of SOS Chromotest procedure

The standard method of Quillardet and Hofnung (1985) recommends a cell concentration of approximately 2×10^7 CFU per mL of assay mixture. Initial experiments were conducted to establish the optimal concentration of cells. Overnight (log phase) PQ37 culture was diluted in fresh LB medium (warmed to 37°C). Where S9 was required, the culture was diluted in 3 parts LB broth and 1 part S9 activation mixture. The sample (or solvent blank), dissolved in 10 μ L DMSO was then added to 190 μ L of diluted culture (plus S9 if desired) present in the first well of each dilution series. The automated multichannel pipette then mixed the well contents and performed a 2-fold serial dilution with an adjacent well that already contained 95 μ L of diluted culture and 5 μ L of DMSO. The two-fold serial dilution was continued in four additional wells for a total of six, 2-fold serial dilutions. This dilution system permits full use of the automated laboratory workstation, while not permitting any change in the concentration of carrier solvent or cells. A minimum of twelve control wells received PQ37 culture and a solvent blank. All extracts examined for genotoxicity were dissolved in pesticide grade DMSO. Each extract was tested in triplicate. The final background concentration of DMSO was always 5% v/v.

Exposure of bacteria and enzyme assays

Experiments were conducted to determine the optimal exposure time. Exposure times of 2, 3 and 4 hours at 37°C were examined. Plates were subsequently centrifuged at 1200g for 20 minutes at 37°C. The supernatant was removed and the bacterial pellet resuspended in 100 μ L of resuspension buffer and 100 μ L of *SOS Chromogen*. Centrifugation and supernatant removal permitted the removal of colored substances that can contribute to the sample's optical density and complicate enzyme activity measurements. Plates were mixed for 60 seconds (Titertek microplate vortex, Flow Laboratories), the time was recorded and the plates returned to the 37°C incubator. A variety of experiments were conducted to determine the optimal time for conversion of the substrates to their colored products. Incubation times of 30, 60, 90, 120 and 150 min were examined. Optical density measurements were taken at 620 nm to measure β -galactosidase activity and 405 nm to measure alkaline phosphatase activity.

Analysis of results

Simultaneous measurements at both 405 nm and 620 nm requires that the measurements of the alkaline phosphatase activity are corrected for the *spillover* of the blue β -galactosidase product (5-Bromo-4-chloro-3-indole) into the 405 nm range (Organics Ltd., 1990). Experiments conducted in our laboratory indicated that the ratio of the optical density of 5-Bromo-4-chloro-3-indole at 405 nm to its optical density at 620 nm is 0.80 ± 0.0050 units. We verified that this ratio is constant for optical density values between 0.02 and 1.3. Therefore, corrected 405 nm measurements (i.e. corrected alkaline phosphatase activity) were calculated as:

$$\text{Corrected OD 405 nm} = \text{Raw OD 405 nm} - (0.80 * \text{OD 620 nm}) \quad (1)$$

Induction of the SOS response pathway at a sample concentration C, denoted R(C), is the ratio of β -galactosidase activity to alkaline phosphatase activity. To account for the background induction of the *sulA* gene, a normalized SOS response induction factor was calculated as $IF = R(C)/R(0)$, where R(0) is the ratio of β -galactosidase to alkaline phosphatase activity averaged over the 12 controls that received blank solvent. A normalized induction factor that exceeded the upper 95% confidence limit of the control was regarded as a significant positive. Since normalized induction factor values are ratios of two means, variance values were calculated according to Welsch et al. (1988)¹. Welsch et al. (1988) describe methods for calculating the variance associated with various functions of mean values (reciprocals, sums, products, ratios, etc). In the case of a ratio, the variance of the ratio is calculated from the mean and variance of the numerator and the denominator as well as their respective reciprocals. Concentration response curves of normalized induction factor against sample concentration (mg sample equivalent per assay mL for SRM extracts and μg per assay mL for pure compounds) were inspected and the results qualitatively categorized as positive if the induction factor exceeded the upper confidence limit of the control at a minimum of three doses.

The genotoxic potency of each sample was assessed via its SOS Response Inducing Potency or SOSIP (Quillardet and Hofnung, 1985). The SOSIP is the initial slope of the concentration-response curve and is expressed as SOS Induction factor units per μg of pure compound or equivalent mg of original SRM. SOSIP values¹ were calculated for each significant, positive response. SOSIP values were determined using ordinary, least squares linear regression (SAS Institute, 1988). In addition, the maximum induction factor obtained was recorded and denoted MaxIF.

¹We used the older, more complicated method of EC Fieller (described in Kendall and Stuart, 1979) to empirically validate this calculation method.

Protocol development

Adaptation of the SOS Chromotest protocol proceeded in 7 separate stages.

- 1) Development of the enzyme assay/cell lysis reagent (*SOS Chromogen*).
- 2) Investigation of the colorimetric interference of complex SRM extracts.
- 3) Automation of the protocol.
- 4) Adjustment of cell density in the assay mixture.
- 5) Determination of optimal exposure time.
- 6) Determination of optimal substrate conversion time.
- 7) Determination of optimal S9 concentration.

Steps 4 through 7 were carried out using both pure substances and extracts of SRMs.

The following abbreviations are used throughout the text: 4-NQO = 4-Nitroquinoline-1-oxide, MNNG = N-Methyl-N'-nitro-N-nitrosoguanidine, MitC = Mitomycin C, NDMA = N-Nitrosodimethylamine, 2-NA = 2-Naphthylamine, BaP = Benzo(a)pyrene, Tris = (Tris[hydroxymethyl])-aminomethane, X-Gal = 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, SRM = Standard reference material, SOSIP = SOS Response Inducing Potency, IF = SOS Induction Factor, MaxIF = Maximum SOS Induction Factor observed, DMSO = Dimethyl sulfoxide, DCM = Dichloromethane, CFU = Colony forming unit, PNPP = *p*-Nitrophenyl phosphate.

RESULTS

The SOS Chromotest microtiter kit contains a ready-to-use *blue chromogen* for the determination of β -galactosidase activity. The *chromogen* uses 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-Gal) as a β -galactosidase substrate. The use of X-Gal, rather than *o*-nitrophenyl- β -D-galactopyranoside (ONPG), permits simultaneous measurement of both β -galactosidase and alkaline phosphatase activity. The composition of this reagent is not provided in any of the documents that accompany the kit. The standard version of the test (Quillardet and Hofnung, 1985) recommends that β -galactosidase and alkaline phosphatase activities be measured in a Tris (Tris[hydroxymethyl])aminomethane or phosphate buffer containing sodium dodecyl sulfate (SDS) to promote cell lysis. In our experience, SDS caused bubbles that interfere with subsequent optical density measurements. Preliminary experiments were conducted to design an effective alternative to the standard enzyme assay and cell lysis reagent. We avoided phosphate buffer since PO_4^{2-} can impair alkaline phosphatase activity (Marzin et al., 1986; Courtois et al., 1988). We replaced the buffer with Super QTM water adjusted to pH 9 with 0.1 N NaOH. The net result is a hypotonic solution that invokes an osmotic shock and causes a sudden expansion of the cell membrane (Neu and Heppel, 1965) against the already fragile (deep rough) cell wall. We replaced SDS with 0.5% v/v toluene. Toluene has been used to disrupt the cell membrane of *Escherichia coli* and permit quantitative determination of β -galactosidase activity (Miller, 1972; Goulding, 1986). The effectiveness of toluene in disrupting the cell membrane is increased by the *rfa* mutation. Deep rough mutants (*rfa*) are highly sensitive to organic solvents by virtue of the fact that they are missing a substantial portion of their outer membrane (Nikaido and Vaara, 1987). The solvent mixture (40% v/v methanol and 10% v/v N,N-dimethyl formamide) effectively dissolves both the enzyme substrates and their products. The final pH of the reagent is between 7.8 and 7.9. This value is very close to the optimal pH values for measurement of both β -galactosidase (7.75) and alkaline phosphatase (8.05) activity (Mersch-Sundermann et al., 1991).

Color interference is a very real problem in the SOS Chromotest. Test compounds and extracts that are strongly colored interfere with the measurements of enzyme activity. This problem is compounded by the poor solubility of many organic substances and extracts in the aqueous assay system. Precipitation of sample components can contribute to sample turbidity. Turbidity, like color, can interfere with the measurement of enzyme activity. Von der Hude et al. (1988) followed the exposure period with a centrifugation step that isolates the exposed bacteria and removes the colored supernatant. Alternatively, an initial optical density measurement, taken immediately after substrate addition, can be subtracted from the

final value (Von der Hude et al., 1988). We explored the centrifugation option, but found that it did not completely remove the colored components of the SRM extracts. In many cases insoluble, colored components remained adhered to the sides and bottom of the microplate wells. Furthermore, initial optical density readings did not effectively compensate for this interference. Optical density readings were often observed to fall for the first 40-60 min after cell resuspension and addition of the SOS Chromogen. This phenomenon is illustrated in Figure 2. The reduction in optical density is presumably caused by a decrease in turbidity resulting from cell lysis and dissolution of organic substances that were insoluble in the original assay medium. In all subsequent experiments initial optical density readings were taken 60 min after cell resuspension and SOS Chromogen addition. Final readings were taken 30-120 min after the initial reading.

Preliminary experiments were conducted to determine the optimal concentration of cells in the assay mixture. The results are illustrated in Figure 3. The results indicate that both the MaxIF and the SOSIP of 4-NQO are highest at 5×10^6 CFU per mL. For the diesel particulate extract, maximum SOSIP and MaxIF were obtained at 1×10^7 CFU per mL. All subsequent experiments were carried out using a cell density of $6-8 \times 10^6$ CFU per mL (30-40% of standard protocol).

Further experiments were carried out to determine the optimal exposure time, substrate conversion time and, for progenotoxic samples, the optimal S9 concentration. Results were interpreted using SOSIP and MaxIF values. Figure 4 illustrates some of the results of experiments to determine an effective substrate conversion time (i.e. enzyme assay incubation time). The results indicate that in some cases substrate conversion time has little effect on the SOS Chromotest results (e.g. MNNG and coal tar extract). Similar results were obtained for 2-NA with S9, Captan with S9 and the urban dust extract with S9. For MitC the highest SOSIP and MaxIF were obtained for substrate conversion times of 30-60 minutes after an initial reading at 60 min. For several other samples net substrate conversion times of 60 min resulted in small (< 3-fold) increases in SOSIP and MaxIF. These samples include 4-NQO, NDMA, and coal tar extract with S9. Substrate conversion time had a particularly dramatic effect on the diesel particulate extract results. The SOSIP associated with a 60 min net incubation time is at least an order of magnitude higher than other values. Overall, the results obtained indicate that for 8 out of the 9 substances tested with or without S9 activation (extract of urban dust elicited an erratic, marginal response in the absence of S9), the SOSIP calculated for a 60 min net substrate conversion time was either higher or not significantly different from that calculated for other incubation times. Figure 5a provides a summary of the effect of substrate conversion time on the mean SOSIP of the complex mixtures examined. Mean values were calculated using all

Figure 2. Time course of SOS Chromogen development at 37°C. Following a 2 hour exposure cells were harvested via centrifugation at 1200g for 20 min. Measurements at 620 nm were taken each 5 min after cell resuspension and SOS Chromogen addition and followed for a total of 180 min. Optical density readings at 620 nm follow the conversion of the β -galactosidase substrate (X-Gal) to its blue product.

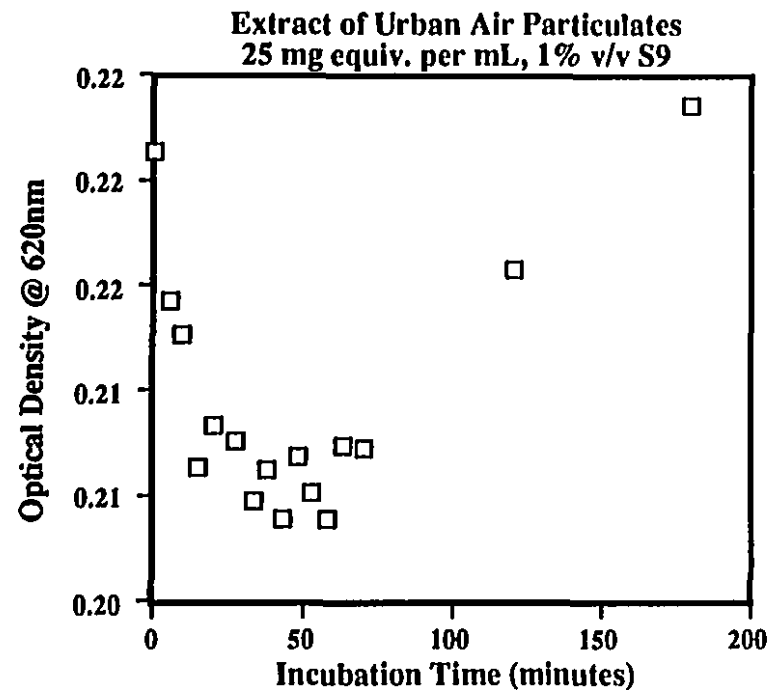
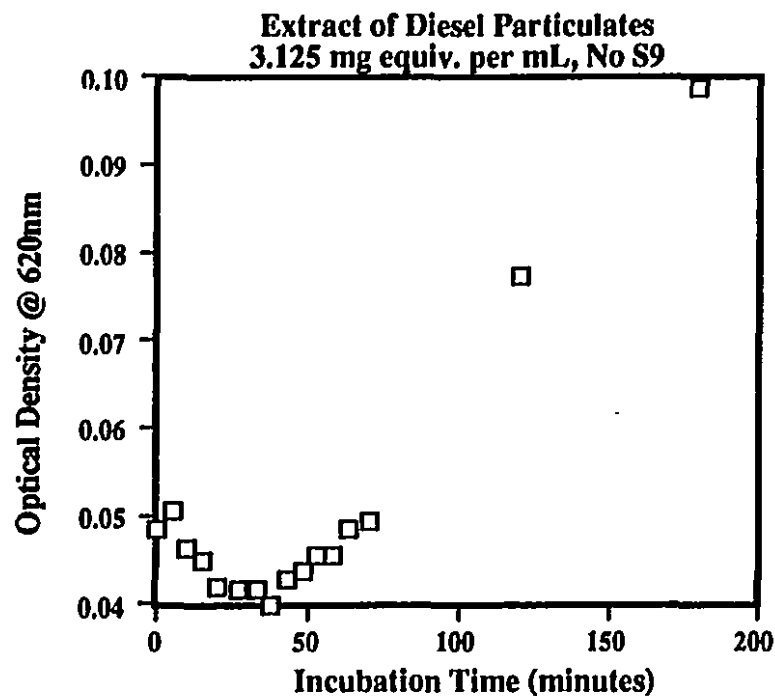


Figure 3. The effect of cell concentration on SOS Chromotest results. The upper panel illustrates the effect of cell density on the SOS genotoxicity of 4-NQO. The corresponding SOSIP values are (lowest to highest cell density): 170.1 IF per μg , 72.5 IF per μg and 32.6 IF per μg . The lower panel illustrates the effect of cell density on the SOS genotoxicity of the diesel particulate extract. The corresponding SOSIP values are: 49.0 IF per mg, 55.7 IF per mg and 15.5 IF per mg. Induction factor values are means of five replicates. Error bars are one standard error of the mean. Where error bars are not shown, they were smaller than the plotting symbol. Exposure time = 2 hr. Substrate conversion time = 120 min total. Cell concentrations were determined by serial dilution and ordinary plate counts on LB agar (20 $\mu\text{g}/\text{mL}$ ampicillin).

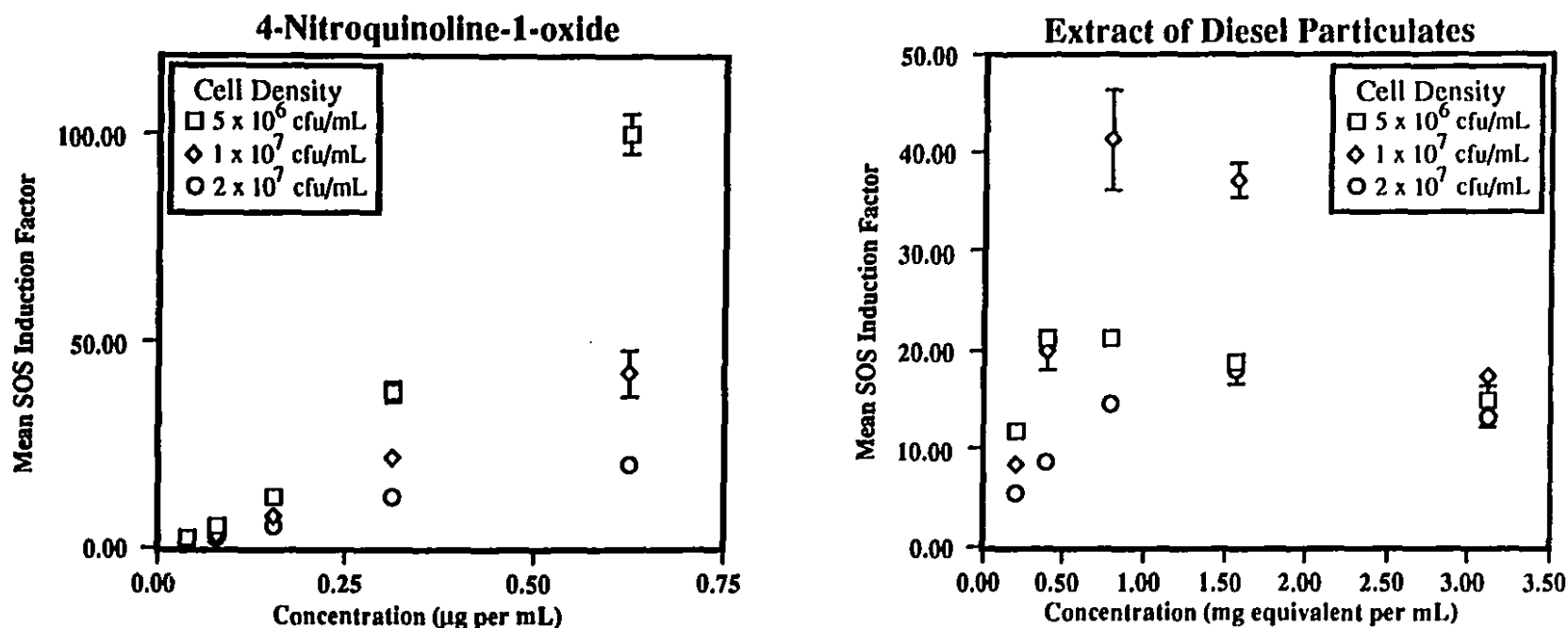


Figure 4. The effect of substrate conversion time on SOS Chromotest results. Exposure time was held constant at 3 hours. In all cases conversion time values indicate minutes *after* an initial measurement taken 60 min after cell resuspension and SOS Chromogen addition. All samples were tested without S9 activation. The two upper panels illustrate the effect of substrate conversion time on the SOS genotoxicity of MitC and MNNG. The corresponding SOSIP values are (shorter to longer incubation time): 1) MitC- 43.5 IF μg^{-1} , 58.2 IF μg^{-1} and 41.9 IF μg^{-1} ; 2) for MNNG- 0.29 IF μg^{-1} , 0.22 IF μg^{-1} and 0.25 IF μg^{-1} . The lower panel illustrates the effect of substrate conversion times on the SOS genotoxicity of the diesel particulate matter extract and the coal tar extract. The corresponding SOSIP values are: 1) diesel particulate extract- 49.1 IF mg^{-1} , 3.6 IF mg^{-1} and 1.2 IF mg^{-1} ; 2) coal tar extract- too few points to calculate SOSIP, values are similar and in 35 IF mg^{-1} range. Induction factor values are means of triplicate samples. Error bars are one standard error of the mean. Where error bars are not shown, they were smaller than the plotting symbol. Initial cell concentration was $6-8 \times 10^6$ CFU per mL.

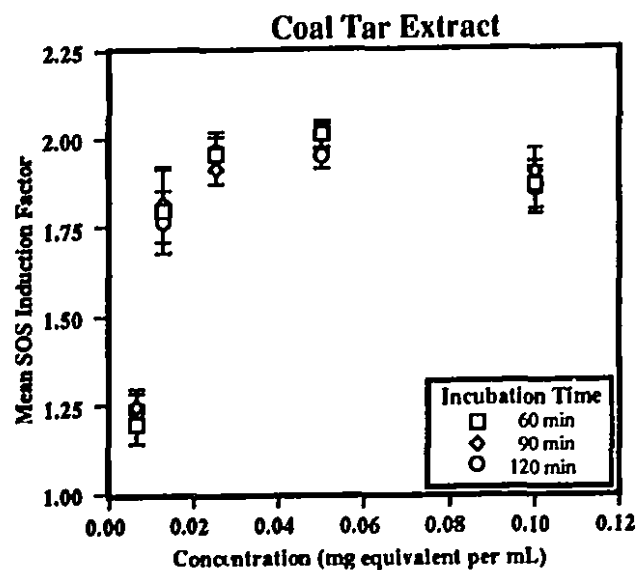
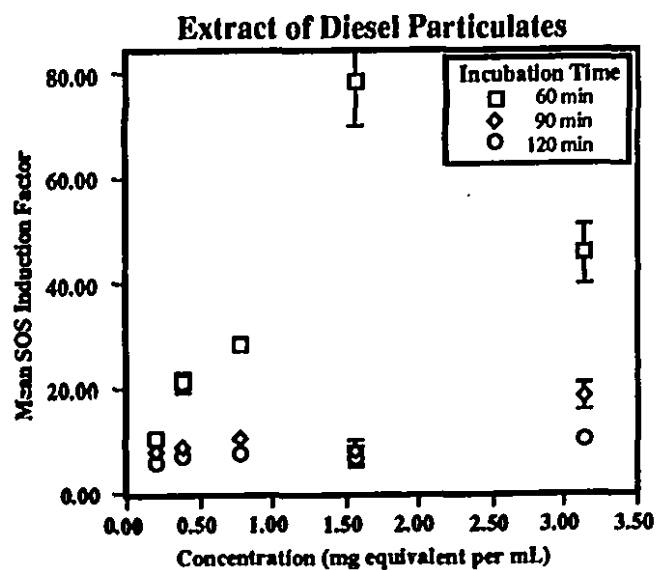
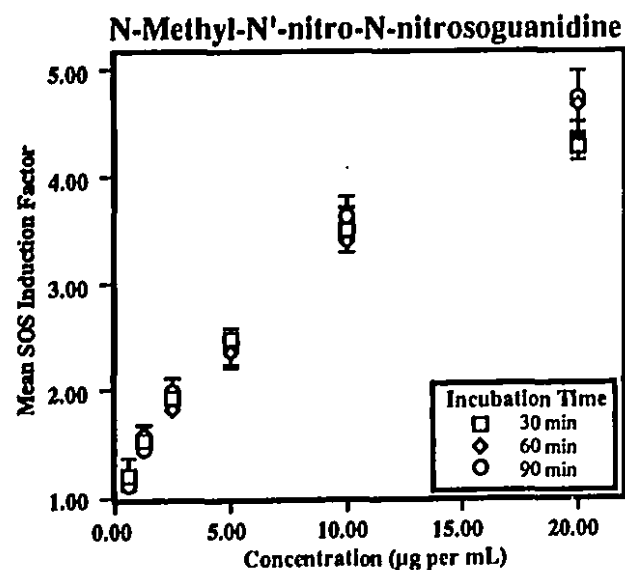
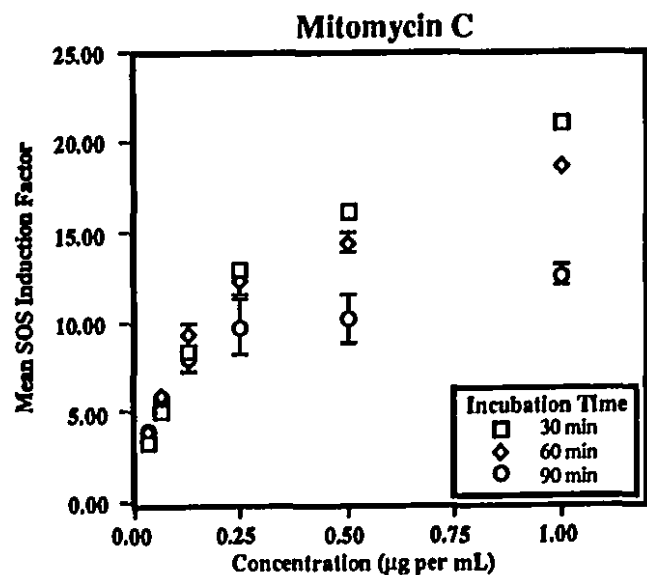
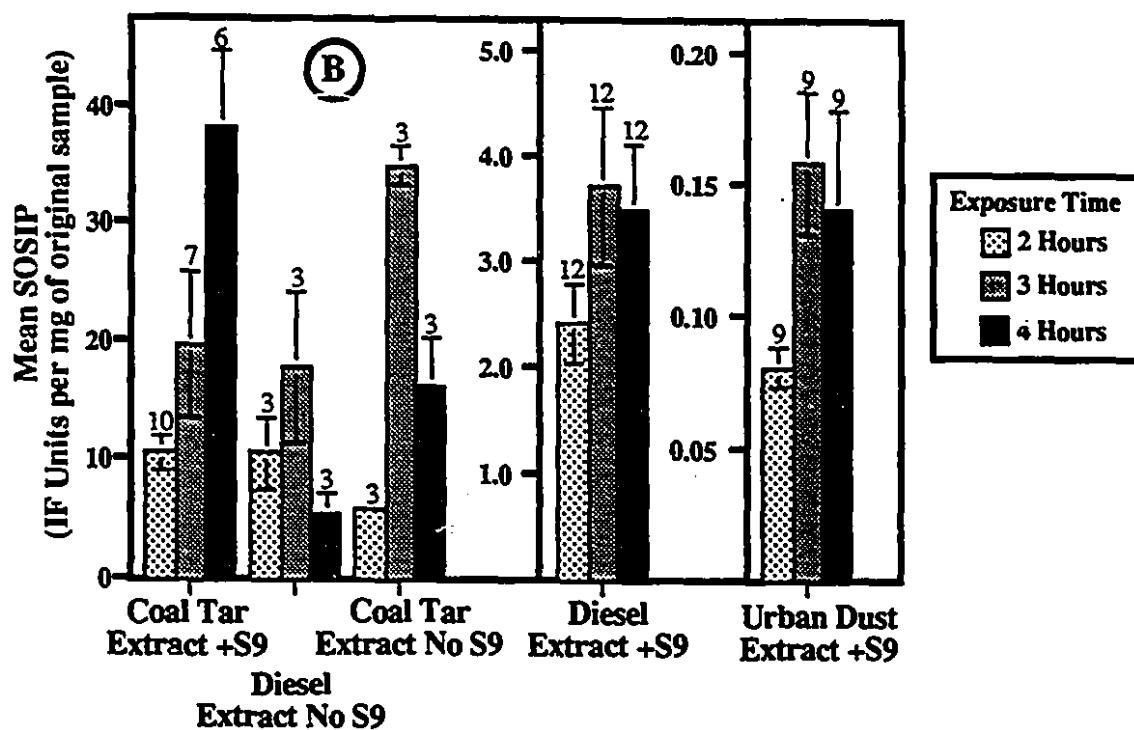
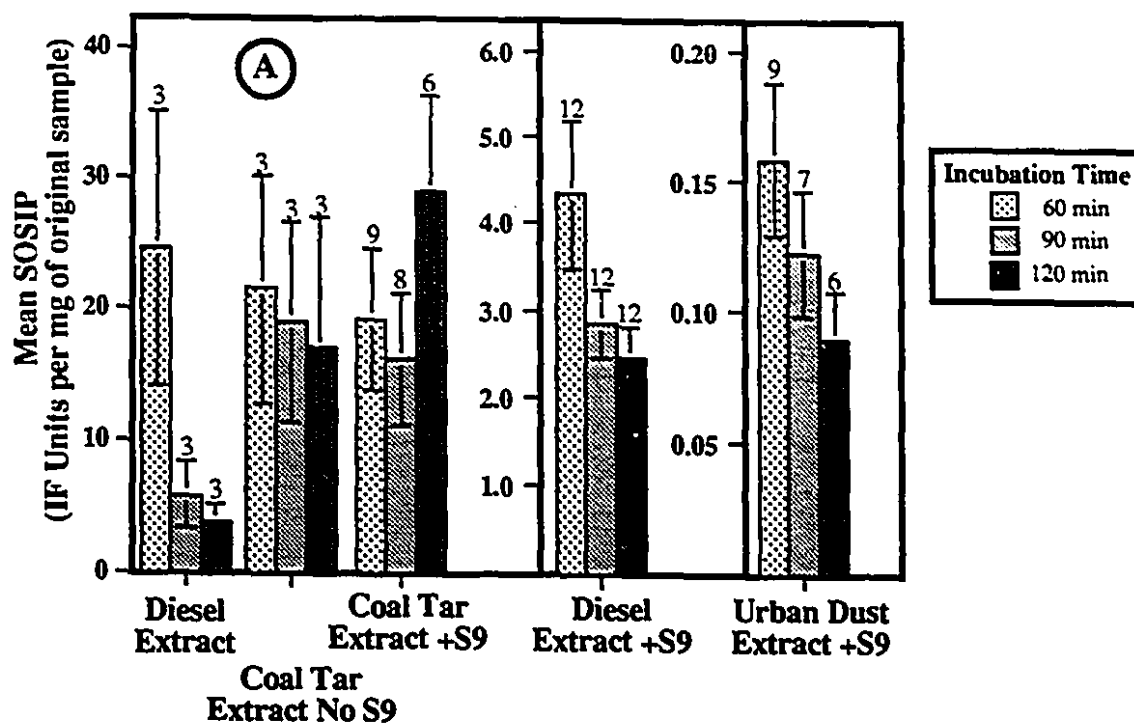


Figure 5. Summary of the effect of exposure time and substrate conversion time on the SOS Chromotest results for 3 complex mixtures. Potency is expressed as mean SOSIP in IF per equivalent mg of original sample. Mean values were calculated from all available experimental data. **A.** The effect of substrate conversion time on the genotoxic potency of coal tar extract, diesel particulate extract and urban dust extract. In all cases incubation times are minutes *after* an initial optical density reading taken 60 min after cell resuspension and SOS Chromogen addition. Error bars are one standard error of the mean. Numbers above error bars indicate sample size. Each individual SOSIP value is based on three concentration-response replicates. **B.** The effect of exposure time on the genotoxic potency of coal tar extract, diesel particulate extract and urban dust extract. Error bars are one standard error of the mean. Numbers above error bars indicate sample size. Each individual SOSIP value is based on three concentration-response replicates.



experimental data. The Figure demonstrates that the highest SOSIPs often occur when the final optical density readings are taken 60 min after the initial reading.

Figure 6 illustrates the effect of exposure time (i.e. contact time between cells and sample) on the SOS genotoxicity of MitC, 4-NQO, diesel particulate extract and coal tar extract. The results indicate that in several instances exposure time has a large effect on genotoxic potency. For diesel particulates without S9 the SOSIP calculated for a 3 hour exposure time is at least 3-fold higher than that calculated for other exposure times. The highest MaxIF for the coal tar extract without S9 was obtained following a 3 hr exposure. Results for diesel particulates with S9 and urban dust with S9 revealed a similar pattern. In addition, the potency of MitC (Figure 6), Captan with S9 and 2-NA with S9 are higher when the exposure time is increased to 3 hours. Exposure time had a relatively minor (< 2-fold) effect on the potency of 4-NQO (Figure 6), MNNG and NDMA with S9. Figure 5b provides a summary of the effect of exposure time on the genotoxic potency of the complex mixtures examined. The figure indicates that in most cases a 3 hour exposure provides the highest mean SOSIP values. The exception is coal tar extract with S9, which elicited a strong response following a 4 hr incubation.

Figure 7 summarizes the effect of S9 enzyme concentration on the SOS genotoxicity of captan, NDMA, 2-NA, diesel particulate extract, urban dust extract and coal tar extract. The results indicate that there is no single S9 concentration that produces the highest genotoxic potency in the majority of samples. Pure substances such as 2-NA elicit a high SOSIP at high S9 concentration (3% v/v). Captan demonstrates the opposite trend (max. at 0.75% v/v). For complex mixtures, the coal tar extract elicits a high SOSIP at high S9 concentrations (4% v/v), while the diesel particulate extract demonstrates the opposite trend (max. at 0.5% v/v). Maximal SOSIP values for the urban dust extract were obtained for S9 concentrations in the 1-2% range.

Based on the results shown in Figures 2 through 7, the following standard protocol was adopted for investigations of complex environmental extracts:

- Cell density = $6-8 \times 10^6$ CFU per mL.
- Exposure time = 3 hours @ 37°C.
- Substrate conversion time (SOS Chromogen incubation time) = 120 min total with initial reading at 60 min.
- Final S9 concentration in assay mixture = 1 % v/v. If results are negative, questionable or erratic repeat with 2-4% v/v.

Figure 6. The effect of exposure time on SOS Chromotest results. *Net* substrate conversion time was held constant at 60 min. All samples were tested without S9 activation. The two upper panels illustrate the effect of exposure time on the SOS genotoxicity of MitC and 4-NQO. The corresponding SOSIP values are (shorter to longer exposure time): 1) MitC- 13.9 IF μg^{-1} , 58.2 IF μg^{-1} and 57.7 IF μg^{-1} ; 2) for 4-NQO- 54.3 IF μg^{-1} , 52.5 IF μg^{-1} and 50.8 IF μg^{-1} . The lower panel illustrates the effect of exposure time on the SOS genotoxicity of the diesel particulate matter extract and the coal tar extract. The corresponding SOSIP values are: 1) diesel particulate extract- 15.7 IF mg^{-1} , 49.1 IF mg^{-1} and 9.0 IF mg^{-1} ; 2) coal tar extract- too few points to calculate SOSIP, highest values appear to correspond to an exposure time of 3 hours (SOSIP in the 35 IF mg^{-1} range). Induction factor values are means of triplicate samples. Error bars are one standard error of the mean. Where error bars are not shown, they were smaller than the plotting symbol. Initial cell concentration was $6-8 \times 10^6$ CFU mL^{-1} .

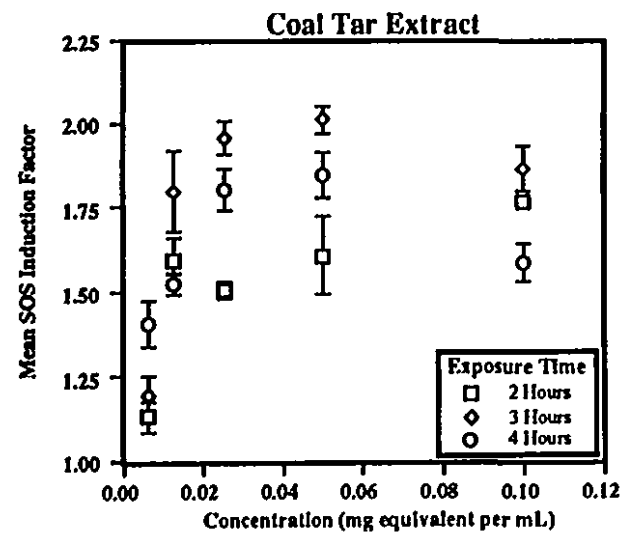
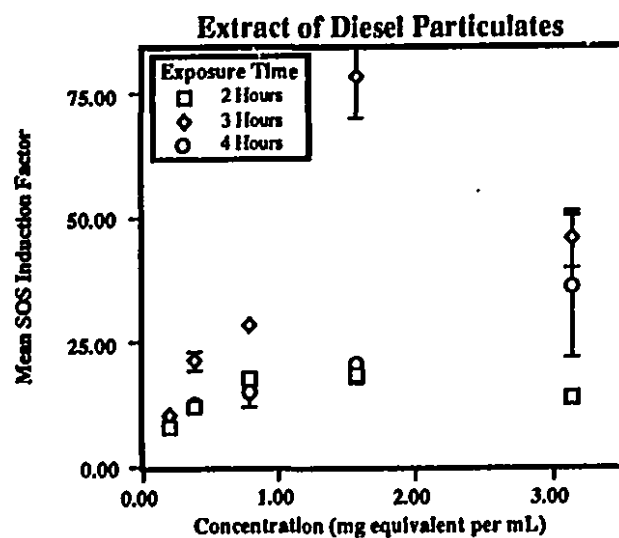
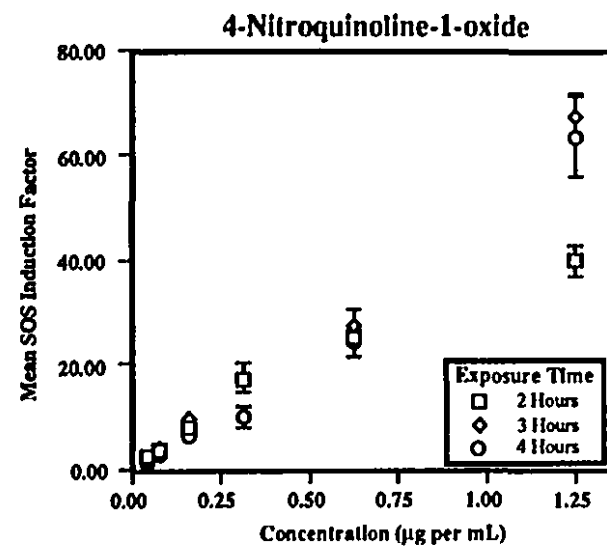
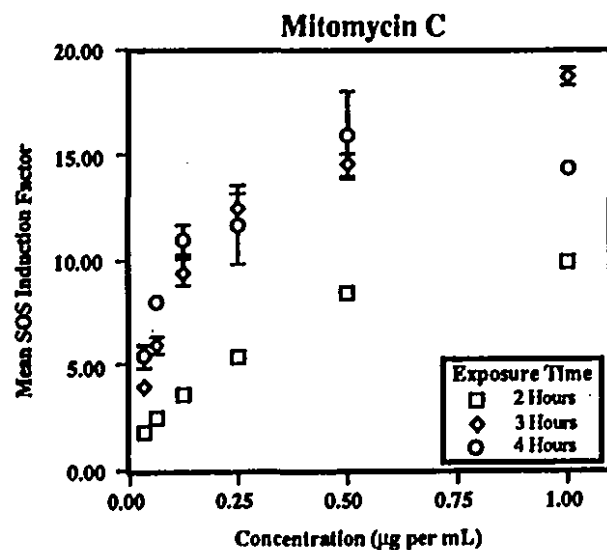
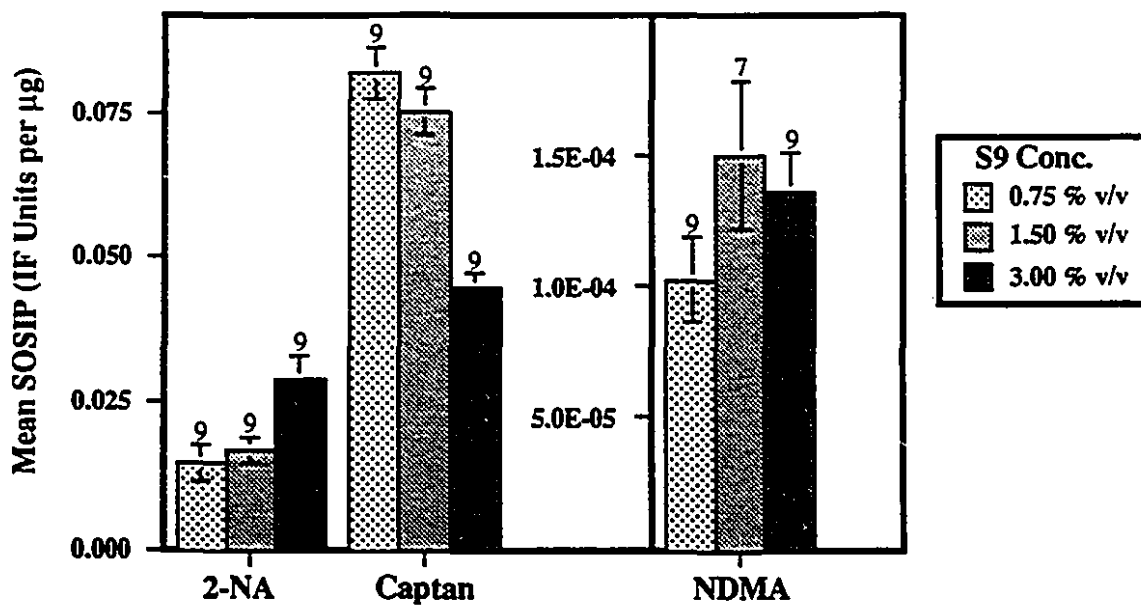
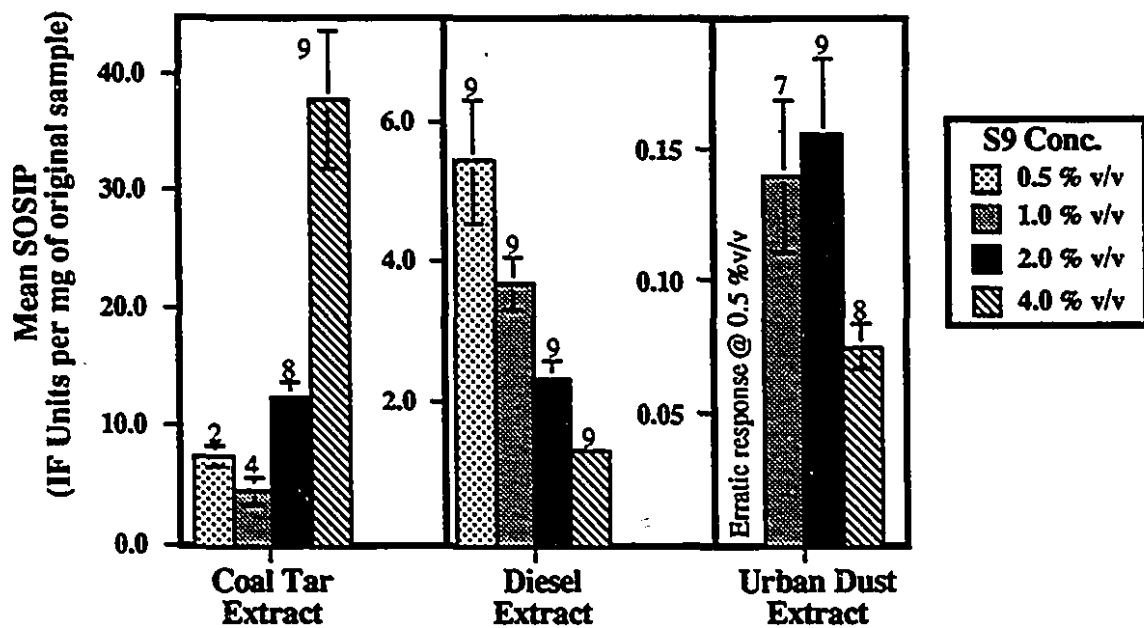


Figure 7. Summary of the effect of S9 concentration on SOS Chromotest results for 3 complex mixtures and 3 reference genotoxins. Potency is expressed as mean SOSIP in IF per equivalent mg of original SRM or IF per μg of pure substance. Mean values were calculated from all available experimental data. The upper panel illustrates the effect of final S9 concentration on the mean SOSIP of 2-NA, Captan and NDMA. The lower panel illustrates the effect of S9 concentration on extracts of coal tar, diesel particulates and urban dust. Error bars are one standard error of the mean. Numbers above error bars indicate sample size. Each individual SOSIP value is based on three concentration-response replicates.

Pure Substances



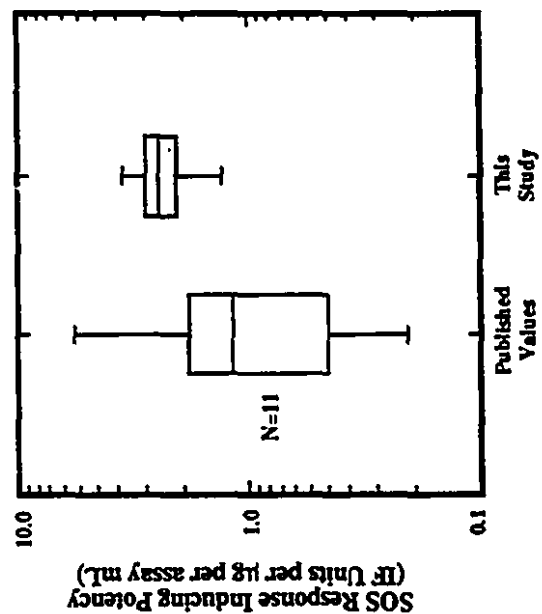
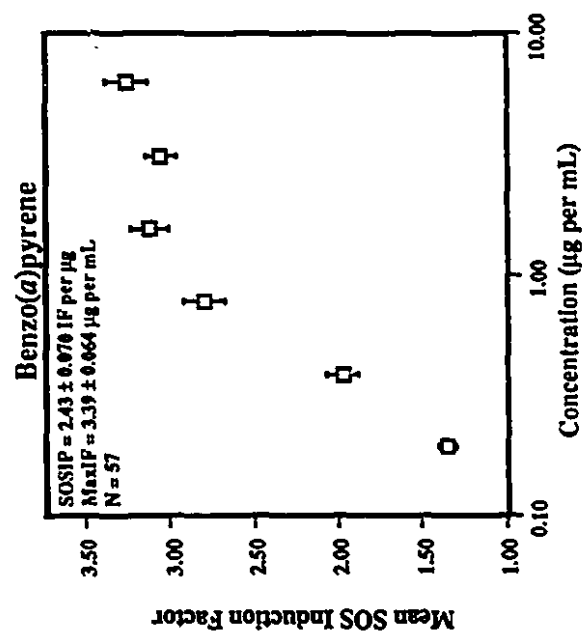
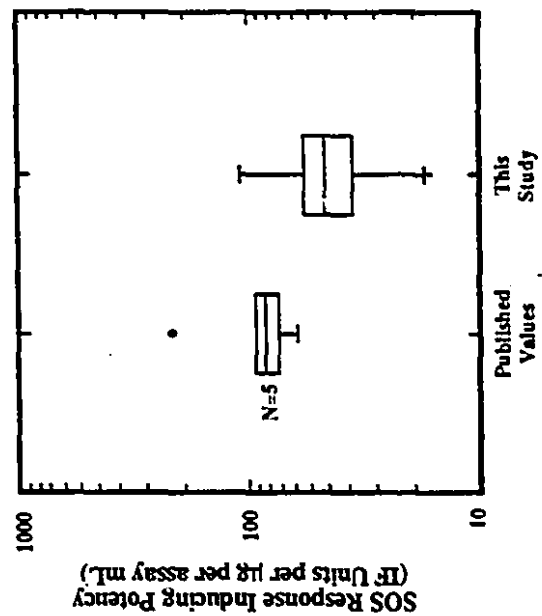
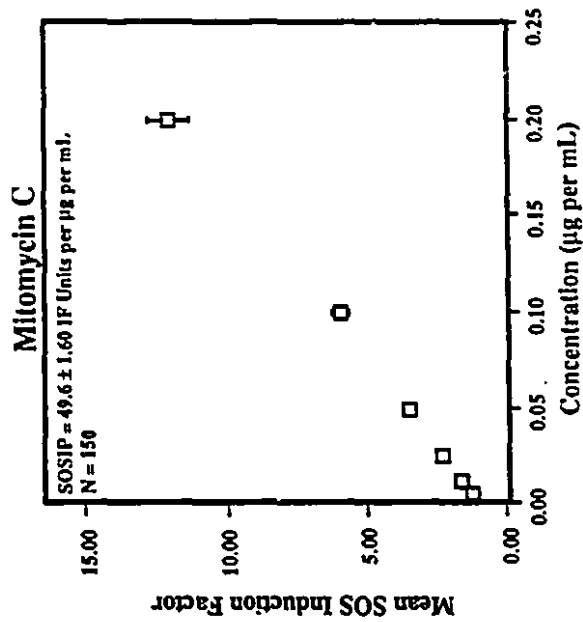
Complex Mixtures



Automation of the modified SOS Chromotest protocol dramatically increased sample throughput. The Biomek™ workstation permitted processing of up to 12 microplates per day. Up to six samples can be tested on each plate, resulting in a maximum daily throughput of 72 samples. This maximum assumes that each sample is tested in duplicate with six, 2-fold serial dilutions and each plate contains 12 solvent control wells and 6 positive control wells. To streamline the assay procedure, 12 microplates were processed in three batches of 4 plates. The automated system is capable of adding the culture media and appropriate controls to a batch of 4 plates in 25-30 minutes. Sample addition and serial dilution requires an additional 15 minutes. Therefore, assay of up to 24 samples can be initiated in under 45 minutes. The completed plates are placed in the 37°C incubator prior to beginning culture addition for the next batch of 4 plates. Separate overnight cultures (staggered by 1 hour) were prepared for each batch of microplates. Culture dilution and S9 mix preparation (where necessary) were carried out immediately prior to assay initiation. Once exposure and centrifugation were completed, cell resuspension, addition of SOS Chromogen and mixing of each microplate requires 3 minutes. Optical density measurements at both 405 and 620 nm require less than 10 seconds per microplate. Including initial and final optical density measurements at both 405 and 620 nm, 12 microplates generate over 4600 optical density values. To handle this quantity of data, we have also partially automated the data analyses. All raw data were processed as a batch using the SAS system version 6.08 (SAS Institute, 1993). We have written several programs that perform all necessary calculations and produce concentration response plots for each tested sample.

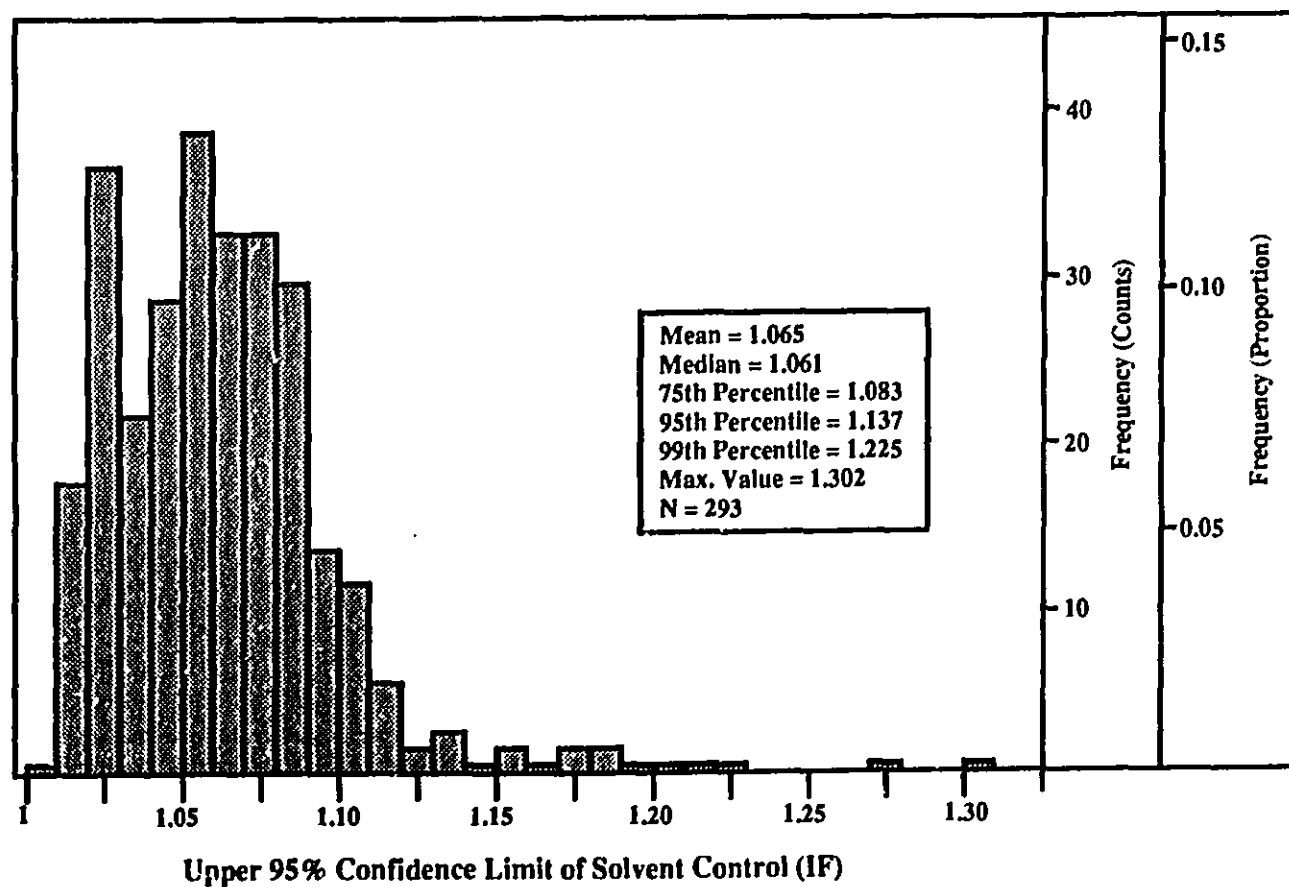
Using the described protocol we have conducted extensive tests on organic extracts of industrial wastes (White et al., 1995b; White et al., 1995c, river sediments (White et al., 1995d) and a wide range of aquatic biota (White et al., 1995e; White et al., 1995f). The large number of assays performed in our laboratory permits an analysis of the long term variability in the response to both positive controls and blank solvent. Figure 8 summarizes the results of 150 microplate assays conducted without S9 and 57 assays conducted with S9. The positive controls were MitC and BaP respectively. The results indicate that the variability in response across microplates is very small. The coefficient of variation on the SOSIP values for BaP and MitC are 2.8 and 3.2% respectively. The lower portion of the figure is a comparison of the SOSIP values obtained using our protocol and values presented in the recent literature. The results indicate that the SOSIP values obtained using the semi-automated, microplate method presented here are very similar to previously published values.

Figure 8. Historical summary of SOS Chromotest results for two reference genotoxins used as positive controls. The SOS Chromotest protocol discussed in the text was used in 150 assays conducted in the absence of S9 and 57 assays conducted in the presence of S9 activation. The upper panels show the concentration-response relationships based on average IF values. Error bars are 2 standard errors of the mean. Where error bars are not shown, they were smaller than the plotting symbol. The lower panels compare the SOSIP values obtained using the protocol described in this study with those presented in the recent literature. Values are summarized using box and whisker plots (Wilkinson, 1987). The horizontal lines dividing the boxes are the median values. The edges of the boxes represent the interquartile range (from the 25th percentile to the 75th percentile). Vertical lines or whiskers extend 1.5 interquartile ranges beyond the box. Values beyond the whiskers are plotted as circles or asterisks. Asterisks represent values 1.5 to 3 interquartile ranges from the box edge. Circles are values more than 3 interquartile ranges from box edge. Literature values for BaP were obtained from: Mersch-Sundermann et al. (1992), Von der Hude et al. (1988), P. Quillardet (pers. comm.), Venier et al. (1989), Mersch-Sundermann et al. (1993), Schleibinger et al. (1989), Tudek et al., (1988), Marzin et al. (1986), Rempola et al. (1986). Literature values for MitC were obtained from: Auffray and Boutibonnes (1987), P. Quillardet (pers. comm.), Venier et al. (1989), Ohta et al. (1984), Rempola et al. (1986).



The variability in the response to the carrier solvent can determine the sensitivity of the assay system. Well behaved control wells, result in tight confidence intervals and an increased ability to detect weak, positive responses. We calculated the upper 95% confidence interval (Welsch et al., 1988; Zar, 1984) on the mean control values from 293 microplate assays conducted in our laboratory. The results obtained are summarized in Figure 9. The results indicate that the upper 95% confidence limit of the solvent control never exceeded 1.302 IF Units and rarely ($\approx 2\%$ of microplates) exceeded 1.20 IF Units. In over 75% of the assays conducted in our laboratory the upper (95%) confidence limit of the solvent control is less than 1.10. These upper confidence limits can be used to calculate minimum genotoxic concentration values (e.g. Langevin et al. (1992) and White et al. (1995a and 1995b)).

Figure 9. Histogram of upper 95% confidence limits on solvent controls. Confidence limits on mean control values were calculated for 293 microplates. Each confidence limit value is based on the results for 12 wells on a given microplate. All solvent control wells contained 5% v/v DMSO. All assays were conducted using the Biomek™ automated laboratory workstation and the SOS Chromotest method described in the text. Variance of mean IF values for solvent controls were calculated according to Welsch et al. (1988). Confidence limits were calculated according to Zar (1984).



DISCUSSION

The SOS Chromotest is a convenient, short-term assay for genotoxicity assessment. Although it has not been used as extensively as the *Salmonella*/microsome assay, it has been successfully used on a wide range of pure substances and complex mixtures. Approximately $\frac{1}{4}$ of the SOS Chromotest publications have examined complex mixtures. Tested samples include bodily fluids and excreta, foodstuffs, industrial wastes, surface waters and sediments and airborne particulate matter. Table 1 summarizes the published studies that used the SOS Chromotest to investigate the genotoxicity of complex samples. These complex samples present several technical challenges for the SOS Chromotest. In overcoming the problems of testing complex mixtures many researchers have modified the original SOS Chromotest protocol. Genotoxins in complex mixtures are frequently present at very low concentrations. As a result, unconcentrated liquid samples frequently elicit weak responses at the detection limit of the assay system (Fish et al., 1987; Dutka et al., 1987; Wong et al., 1994). Many researchers have used extraction/concentration procedures to overcome this problem (De M  o et al., 1988; Venier et al., 1989; Kohn et al., 1988; Langevin et al., 1992; Pfeil et al., 1994; White et al., 1995a). Solid samples are usually physically incompatible with the aqueous assay. These samples are usually extracted with organic solvent (Courtois et al., 1988; White et al., 1995b; Langevin et al., 1992; McDaniels et al., 1993; Poirier et al., 1989). Resulting organic extracts as well as oily samples are not easily mixed with the aqueous medium (Raabe et al., 1993; White et al., 1995b). Some researchers have attempted to use nonionic detergents to overcome this problem (Janz et al., 1990; Raabe et al., 1993; Braun et al., 1993). Turbid and colored samples interfere with spectrophotometric measurements (Langevin et al., 1993; White et al., 1995b; Venier et al., 1989; Hoflack et al., 1993; Lan et al., 1991). This problem is often overcome by post-exposure centrifugation and supernatant removal (Bosworth and Venitt, 1986; White et al., 1995b; Legault, pers. comm.) and/or initial optical density readings taken immediately after addition of the enzyme assay reagent(s) (Wong et al., 1994; McDaniels et al., 1993; Lan et al., 1991; Nair et al., 1990; Venier et al., 1989; Poirier et al., 1989).

In addition to protocol modifications that permit the testing of complex mixtures, additional protocol modifications have been introduced. The majority of these concern culture methods, concentration of cells in the assay mixture, incubation times, the concentration of S9 and cofactors in the activation mixture and the make-up of the cell lysis/enzyme assay reagent. The net result is a wide range of SOS protocols for the analysis of complex samples, few of which have been validated in any way. Several researchers

Table 1. Summary of published studies which used the SOS Chromotest to investigate the genotoxicity of complex mixtures.

Sample Studied	Results Obtained	Source
1) Urine and Urine Concentrates		
Unconcentrated urine.	Positive response for subjects exposed to toxic mineral oils.	1
XAD-2 [†] extract.	No positive response.	2
XAD-2 and C ₁₈ [§] SEP-PAK extracts.	Positive response for patients receiving antineoplastic drugs (+S9 where required).	3
XAD-2 extract.	Positive response (+S9 and -S9) for patients receiving coal tar treatment.	4
2) Airborne Particulates		
DCM [‡] and acetone extracts of samples from Paris area.	Several positive responses. No response in presence of S9.	5
DCM extracts of samples from Berlin area.	Several positive responses. Higher activity with S9.	6
DCM extracts of samples from Gangzhou, China.	Several positive responses without S9.	7
3) Industrial Wastes and Effluents		
Unconcentrated effluents from 7 industries.	Several positive responses. Algal activation documented.	8
Several waste samples.	Investigated the effect of detergents on the response of complex mixtures.	9
Unidentified industrial waste.	Effect of complex waste on 4-NQO response.	10
Solid waste from semiconductor manufacture.	Several positive responses (PQ37 and PQ243) both with and without S9.	11
Aqueous soil and waste leachates.	Positive responses without S9.	12
XAD-8, XAD-4 extracts of bleached Kraft mill effluent.	Positive response on several fractions (both with and without S9).	13

Table 1 Continued.....

Kraft pulp spent liquors (pulp and paper waste).	Several positive responses without S9. No response for hardwood pulps.	14
DCM extracts of final effluents from 42 industries.	Samples of varying potency. S9 frequently caused reduction in potency.	15
DCM extracts of effluent suspended particulates.	High potency positive responses (relative to aqueous filtrate).	16

4) Surface Waters, Sediment and Soils

Surface water and aqueous sediment extracts.	Several positive responses on surface waters and sediments (with S9).	17
DMSO extracts of marine sediments.	Several positive responses both with and without S9.	18
DMSO extracts of river sediment.	Several positive responses with S9.	19
DCM extracts of surface water and sediments.	Positive responses on water, suspended and bottom sediments (both +S9 and -S9).	20
Unaltered river sediments.	Direct sediment test procedure (DSTP).	21
DCM and cyclohexane extracts of contaminated soils.	Several positive responses both with and without S9.	22
C ₁₈ extracts of groundwater.	Weak, but detectable responses at several sites (without S9).	23
Raw surface waters and sediment extracts (aqueous and DMSO).	Several positive responses on water and bottom sediment extracts without S9.	24

5) Miscellaneous Samples

Orange juice.	Detected Aflatoxin B ₁ genotoxicity in juice.	25
Cow's milk.	Aflatoxin M ₁ not detected in milk extract.	26
Various complex mixtures.	Discussed effects of extraction solvents on test results.	27
Aqueous extract of faeces.	No clear positive response. Exogenous catalytic activity documented.	28
Aqueous extract of faeces.	No positive response.	29

Table 1 continued.....

XAD-2 extract of red wine.	Weak positive responses both with and without S9.	30
Refined hard-wood smoke flavor.	No positive response with or without S9.	31
Aqueous and organic extracts of preserved foods.	Several weak, positive responses without S9.	32
Ethyl acetate extract of faeces.	Positive responses without S9.	33
Aqueous extract of medicinal plant.	Positive response on SOS spot test.	34
DCM extracts of SRM 1650 and SRM 1649.	Strong, positive response to SRM 1650. Questionable response to SRM 1649.	35
DCM extracts of melted snow.	Several positive samples. Most required S9 activation.	36

Literature cited: (1) Fish et al., 1987; (2) De M  o et al., 1988; (3) Kohn et al., 1988; (4) Venier et al., 1989; (5) Courtois et al., 1988; (6) Schleibinger et al., 1989; (7) Quian et al., 1994; (8) Harwood et al., 1989; (9) Raabe et al., 1989; (10) Janz et al., 1990; (11) Braun et al., 1993; (12) Hoflack et al., 1993; (13) Rao et al., 1994; (14) Nylund et al., 1994; (15) White et al., 1995b; (16) White et al., 1995c; (17) Dutka et al., 1987; (18) Xu et al., 1987; (19) Lan et al., 1991; (20) Langevin et al., 1992; (21) Kwan and Dutka, 1992; (22) McDaniels et al., 1993; (23) Pfeil et al., 1994; (24) Wong et al., 1994; (25) Riesenfeld et al., 1985; (26) Fremy and Quillardet, 1985; (27) Carver and Machado, 1986; (28) Bosworth and Venitt, 1986; (29) Venitt and Bosworth, 1986; (30) Rueff et al., 1986; (31) Jenek et al., 1988; (32) Poirier et al., 1989; (33) Nair et al., 1990; (34) Vargas et al., 1990; (35) Nylund et al., 1992; (36) White et al. (1995a)

†Non-polar polystyrene resin (Amberlite®).

§Non-polar, bonded-phase extraction cartridge.

‡Dichloromethane.

have mentioned that although the SOS Chromotest is convenient and practical, its use on complex mixtures requires protocol validation (Courtois et al., 1988; Nylund et al., 1992) such as that performed by Whong et al. (1986) for the SOS/*umu* assay. The goal of this work was to produce a rapid, cost-effective microplate version of the SOS Chromotest and validate its performance on several complex samples.

The protocol described here includes several modifications of the standard SOS Chromotest protocols (Quillardet and Hofnung, 1985; Orgenics Ltd., 1990; Fish et al., 1987, Mersch-Sundermann et al., 1991). The SOS Chromogen advocated does not contain SDS and contains both β -galactosidase (X-Gal) and alkaline phosphatase (PNPP) substrates in a solvent mixture that promotes cell lysis. Several researchers have recommended modification of the *SOS Chromogen* or enzyme assay/cell lysis reagent (Mersch-Sundermann et al., 1991; Marzin et al., 1986; Nair et al., 1990). However, for the most part the enzyme assay reagent has remained unchanged. The removal of SDS avoids bubbles and possible detrimental effects on β -galactosidase. De M  o et al. (1988) and Whong et al. (1986) demonstrated that SDS concentrations as low as 0.1% can reduce β -galactosidase activity by 1-2% per minute. Addition of both X-Gal and PNPP to the SOS Chromogen is advantageous since it permits the simultaneous measurement of both β -galactosidase and alkaline phosphatase activity (Fish et al., 1987).

We advocate the use of centrifugation to remove the tested substance prior to enzyme activity measurements. While initial optical density measurements taken immediately after SOS Chromogen addition can correct for the color or turbidity of the tested substance or extract, it cannot correct for interference with enzyme activity. Several researchers have documented specific inhibition of β -galactosidase and/or alkaline phosphatase. Courtois et al. (1988 and 1992) and Marzin et al. (1986) have demonstrated that components of the S9 activation mixture can passively inhibit β -galactosidase. Olivier and Marzin (1987), Hoflack et al. (1993) and Venitt and Bosworth (1986) demonstrated that some substances (e.g. AlCl_3 , landfill leachates, faecal extracts) can alter the activity of β -galactosidase and/or alkaline phosphatase. In some cases such alterations can likely be attributed to the presence of metal ions (e.g. Cu^{2+} , Zn^{2+} , Hg^{2+} , Pb^{2+} , Ni^{2+}) that are known to inhibit β -galactosidase (Cohn and Monod, 1951; Biswas, 1987) and alkaline phosphatase activity (Asthana et al., 1992; Rueter, 1983). Differential, post-exposure inhibition of either β -galactosidase or alkaline phosphatase by the tested substance will result in ambiguous results. The results presented (Figure 2) indicate that after addition of the SOS Chromogen the optical density values reach a minimum after approximately 40-60 minutes. This initial drop is likely caused by cell lysis and dissolution of colored substances adhered to the side and bottom of the microplate well. For this reason we advocate centrifugation and

supernatant removal followed by initial optical density readings taken 60 min after cell resuspension Chromogen addition.

Recent publications indicate that recommended cell concentrations in the SOS Chromotest assay mixture vary about 5-fold from $\approx 4 \times 10^6$ cells per mL (e.g. Janz et al., 1989) to $\approx 2 \times 10^7$ cells per mL (e.g. standard procedure of Quillardet and Hofnung, 1985). Both Mersch-Sundermann et al. (1991) and Janz et al. (1989) observed that although reductions in cell number cause reductions in enzyme activity, induction of the SOS response (measured by IF and SOSIP values) is increased. In addition, Janz et al. (1988) found that the variability in SOS induction factor values was minimized at cell densities in the 4×10^6 cells per mL range. The results obtained here also indicate that lower cell concentrations in the assay mixture can increase SOSIP values. For routine testing of complex mixtures, we advocate from 6×10^6 to 8×10^6 cell per mL of assay mixture. In addition, like Mersch-Sundermann et al. (1991), we recommend culturing of bacteria in glassware that provide a large surface area for gas exchange and dilution of overnight cultures (15 hr max.) in pre-warmed broth.

SOS Chromotest publications recommend a variety of exposure times. The standard procedure of Quillardet and Hofnung (1985) as well as the Orgenics Ltd. microplate kit procedure and the Bioscreen Analyzer[®] microplate procedure (Janz et al., 1988) recommend an exposure time of 2 hrs at 37°C. The majority of published complex mixture studies (e.g. Xu et al., 1987; Lan et al., 1991; Wong et al., 1994; Venitt and Bosworth, 1986) employed a 2 hour exposure period. Mersch-Sundermann et al. (1991) and Quillardet and Hofnung (1985) determined that, with respect to several pure substances, there is no reason to expose PQ37 for longer than 90 min. However, they both acknowledge that in some instances exposure times greater than 2 hrs may be required for maximum SOS response induction, particularly when cell density is reduced. Nair et al. (1990) and Pfeil et al. (1994) employed a 3 hour exposure period in their study of faecal (Nair et al.) and groundwater (Pfeil et al.) extracts. The results obtained in this study indicate that optimal results are frequently obtained following a 3 hr exposure (see Figures 5b and 6). It is possible that microplate methods require longer incubations due to the reduced aeration of microplate wells compared with that of agitated 15 mL glass tubes (standard method of Quillardet and Hofnung, 1985).

Recent publications indicate that recommended substrate conversion times vary from 10 min to 180 min. This variability can likely be attributed to variations in the composition of the SOS Chromogen and its concomitant efficacy for cell lysis and substrate conversion. The standard tube method of Quillardet and Hofnung (1985) uses ONPG as a β -galactosidase substrate and recommends 10-90 min incubation with the SOS Chromogen.

Lan et al. (1991) indicated that optimal results (highest IF values) were obtained after 60 min incubation with SOS Chromogen. Xu et al. (1987) observed that optimal results are frequently obtained when a 90 min incubation time is employed. Poirier et al. (1989) employed a 120 min incubation time for measurement of β -galactosidase activity. Mersch-Sundermann et al. (1991) examined incubation times up to 60 min and concluded that SOS Chromogen incubation time has a negligible effect on test performance. They recommend a 25 min incubation. It appears that SOS Chromogen mixtures containing X-Gal require longer incubations. However, recommended times vary considerably. The standard kit method recommends an incubation of 60-90 minutes. McDaniels et al. (1990) recommended an incubation time of 120 min. Legault et al. (1994) recommend 60 min without S9 and 90 min in the presence of S9. Nair et al. (1990) describe a modified SOS Chromogen and recommend incubation for 3 hours. In this study we determined that optimal results (high SOSIP and high MaxIF) are frequently obtained when the final optical density reading is taken 60 min following an initial reading taken 60 min after Chromogen addition. For some samples, such as the diesel particulate extract, the Chromogen incubation time had a large effect on the calculated SOSIP (see Figures 4 and 5a). For other samples (both pure substances and complex mixtures) the net Chromogen incubation time had a small or negligible effect on SOSIP and MaxIF. Recent genotoxicity analyses of 150 industrial effluent extracts (White et al., unpublished) revealed that net Chromogen incubation times of more than 60 min are rarely required for optimal results.

We also investigated the effect of S9 enzyme concentration on the SOS genotoxicity of three pure substances and three complex mixtures. Published SOS Chromotest results advocate a wide range of S9 concentrations. The standard method of Quillardet and Hofnung (1985) recommends a final S9 concentration in the assay mixture of 9% v/v. However, Courtois et al. (1988), Marzin et al. (1986) and Mersch-Sundermann et al. (1993) have determined that when used at high concentration, S9 enzymes can inhibit the activity of β -galactosidase. For optimal results, Mersch-Sundermann et al. (1993) recommend final S9 concentrations in the 1.8 to 4.5% v/v range (20-50% of the standard method). Researchers studying both complex mixtures and pure substances often recommend S9 concentrations far lower than that originally recommended by Quillardet and Hofnung (1985). Poirier et al. (1989) used several concentrations of S9 between 0.9 and 18% v/v in their examination of preserved food extracts. Nylund et al. (1992), McDaniels et al. (1993), De M  o et al. (1988), Langevin et al. (1992), Nylund et al. (1994), White et al. (1995a) and White et al. (1995b) used < 2% v/v to examine complex extracts of standard reference materials, contaminated soil, human urine, surface water and sediment, melted snow and industrial effluents. This concentration is close to the concentration

recommended by Maron and Ames (1983) for the plate incorporation version of the *Salmonella* test (20-50 μ L S9 per plate or $\approx$ 0.74-1.85% v/v). The results obtained here indicate that there is no single concentration of S9 that is appropriate for all tested substances. For complex mixtures the optimal S9 concentration ranged from 0.5% to 4% v/v (see Figure 7). Similar results have been obtained for the *Salmonella* test (Courtois et al., 1992). As a result several researchers (e.g. Venitt et al., 1985; Ashby, 1986; Marzin et al., 1986) recommend that tests be carried out at several S9 concentrations.

The results obtained indicate that the potency of the diesel particulate extract is inversely related to the final S9 concentration (see Figure 7). Nylund et al. (1992) and Savard et al. (1992) also observed that the addition of S9 can cause large decreases in the mutagenic potency of diesel particulate extracts (SRM 1650). Many other researchers (e.g. Møller et al., 1982 and Saleem et al., 1984) have documented the direct-acting genotoxicity of diesel exhaust particulates. Our results further indicated that although the coal tar extract (SRM 1597) is genotoxic in the absence of S9 enzymes, the maximal response was obtained in the presence of 4% v/v S9. The IPCS collaborative study on complex mixtures did not obtain a positive *Salmonella* test response for SRM 1597 in the absence of S9 activation (Claxton et al., 1992). However, Goto et al. (1992) obtained a weak positive response on *Salmonella* TA98 without S9 and Whong et al. (1986) obtained a positive response without S9 for a coal dust extract examined using the SOS/*umu* test. Finally, our results indicated that in the absence of S9 activation, the urban dust extract (SRM 1649) was highly toxic and was an extremely weak inducer of the SOS response (MaxIF < 1.4). The optimal response for SRM 1649 was obtained at an S9 concentration of 1-2% v/v. Although organic extracts of airborne particulates frequently demonstrate enhanced potency in the presence of S9 enzymes (Schleibinger et al., 1989; Møller et al., 1982; Pitts et al., 1982; Matsumoto and Inoue, 1987), Courtois et al. (1988) indicated that extracts of particulates collected in Paris invoked the SOS response only in the absence of S9. The IPCS collaborative study determined that the mutagenic potency of SRM 1649 extracts on *Salmonella* TA98 and TA100 was not substantially altered when S9 was added.

At the present time we have used the described protocol for almost 300 microplate assays. The results obtained for 150 assays with Mitomycin C and 57 assays with benzo(a)pyrene indicate that SOSIP variability is very low. The low standard error of mean SOSIP values can be attributed to the precision and accuracy of the automated Biomek™ system. Biomek™ precision and accuracy also reduced the variability of the solvent controls. Our results indicate that the upper 95% confidence limit of the solvent control is rarely above 1.20 SOS IF Units. The original SOS Chromotest protocol of Quillardet and Hofnung (1985) recommends that only samples which invoke induction factors above 1.5

be denoted positive. Although some researchers also require evidence of a dose-related response (Nylund et al., 1992; Legault et al., 1994; Hoflack et al., 1993; Rao et al., 1994; Quillardet and Hofnung, 1993; Poirier et al., 1989), most only categorize induction factors above 1.5 as "significant" positives (e.g. Legault, 1994; von der Hude et al., 1988; Braun et al., 1993; Venier et al., 1989; Nylund et al., 1992; Poirier et al., 1989). Mersch-Sundermann et al. (1992) only considered induction factors greater than 2.0 as "significant" positives. Other researchers have reduced this threshold to 1.3 (Wong et al., 1994; Dutka et al., 1987; Kwan and Dutka, 1992). An alternative to an arbitrary threshold value is a statistical comparison of response IF values to the mean IF of the solvent control (Xu et al., 1989; Lan et al., 1991; Langevin et al., 1992; White et al., 1995a). While this approach is less subjective, it requires that the standard error of the control be calculated correctly. We have routinely used the method of Welsch et al. (1988) to calculate the standard error of the solvent control (White et al., 1995b). Our results (see Figure 9) indicate that the somewhat arbitrary value of 1.5 is unnecessarily high. At the present time we routinely categorize SOS Chromotest results as follows:

- 1) Negative- induction factor never exceeds the upper confidence limit of the control.
- 2) Marginal- induction factor exceeds the upper confidence limit of the control at one or two concentrations only.
- 3) Positive- induction factor exceeds the upper confidence limit of the control at a minimum of three doses.
- 4) Erratic- induction factor may exceed the control at three or more concentrations, but the concentration-response relationship is highly erratic and can not be interpreted.

Effective assessment of the potential genotoxic hazard posed to humans and other biota by complex industrial wastes requires that large numbers of samples be examined. Efficient processing of these samples requires a bioassay system that is rapid, efficient, cost-effective and sensitive. We have described a semi-automated, microplate version of the SOS Chromotest for the analysis of complex environmental extracts. We have exploited the rapid growth rate of *Escherichia coli* and the aforementioned advantages of the SOS Chromotest to produce a protocol that can process up to 72 samples in a single working day. We have already used this method to analyze over 750 complex environmental extracts. Although we have not performed any simultaneous comparisons between the SOS Chromotest and the *Salmonella* test, some researchers claim that the SOS Chromotest is more sensitive for the detection of genotoxicity in complex mixtures (McDaniels et al., 1993; Lan et al., 1991; McDaniels et al., 1990). The use of the Biomek™ automated

laboratory workstation permitted increased accuracy, precision and throughput. While the Biomek™ is a specialized piece of laboratory equipment, it is important to realize that it is not dedicated to performing the SOS Chromotest. The instrument is readily available and can carry out the reagent transfers and dilutions required for a wide range of bioassays and other laboratory procedures. In our laboratory it has also been used to perform a sub-lethal bioassay with the green alga *Selanastrum capricornutum*. While the instrument is expensive (≈ \$25 000 US), it is no more costly than many other laboratory instruments (e.g. HPLC, ultracentrifuge, scintillation counter, fluorescence spectrophotometer, etc).

ACKNOWLEDGEMENTS

We gratefully acknowledge the support and assistance of several people. Philippe Quillardet and Maurice Hofnung of the Pasteur Institute (Paris) for supplying *Escherichia coli* PQ37 and raw SOS Chromotest data. Chantale Côté, Manon Harwood and Richard Legault for comments and discussions on SOS Chromotest methodology. Raymond Vezeau for supplying access to Environment Canada laboratory facilities. Beckman Instruments (Canada) Inc. for providing technical assistance and Biomek™ labware. The project was funded by a St. Lawrence Centre - Natural Sciences and Engineering Research Council of Canada research partnership grant to JBR and a Fondation Canadienne d'Aide à la Recherche grant to PAW.

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CHAPTER 2

**SOS CHROMOTEST RESULTS IN A BROADER CONTEXT.
EMPIRICAL RELATIONSHIPS BETWEEN GENOTOXIC
POTENCY, MUTAGENIC POTENCY AND CARCINOGENIC
POTENCY**

INTRODUCTORY REMARKS

As with all (micro)bioassays, the implications of a positive response on the SOS Chromotest are not necessarily obvious. Those researchers who are not familiar with SOS genotoxicity frequently request that the meaning and implication of a positive response be clarified. This chapter explores the empirical relationships between SOS genotoxicity and two familiar endpoints- Ames/*Salmonella* mutagenicity and animal (primarily rodent) carcinogenicity. It provides predictive models that permit SOS Chromotest results in subsequent chapters to be compared with the large body of *Salmonella* mutagenicity data and animal carcinogenicity data.

The material presented has been accepted, with revisions, for publication in *Environmental and Molecular Mutagenesis*. Prof. Joseph Rasmussen helped to develop many of the ideas presented. He is a co-author on the manuscript.

Appendix 4 contains the data used to generate all models, figures and tables.

ABSTRACT

Environmental monitoring requires that large numbers of samples be processed in a relatively short period of time. While microbioassays facilitate rapid testing, the results are often difficult to interpret in the broader context of human or animal health. Determining the consequences of exposure to genotoxic substances will ultimately require *in situ* monitoring of exposed organisms. However, it is immediately possible to construct a broad empirical framework within which available microbioassay results can be interpreted. To do this for SOS Chromotest results, we investigated the empirical relationships between SOS genotoxic potency and mutagenic potency (as measured with the *Salmonella*/microsome assay), as well as between genotoxic potency and carcinogenic potency (as measured using standard, chronic animal bioassays). Strong relationships were identified between: 1) genotoxic potency and mutagenic potency for 256 direct acting substances, and 2) genotoxic potency and mutagenic potency for 122 S9 activated substances. Analysis of covariance (ANCOVA) of the SOS genotoxicity-Ames mutagenicity relationship revealed that the SOS genotoxicity effect covaries with the *Salmonella* strain used to measure mutagenic potency. With one exception, the results did not reveal significant interactions between SOS genotoxic potency (SOSIP) and *Salmonella* strain. The ANCOVA models relating SOS genotoxicity and Ames mutagenicity were used to predict the mutagenic potency of several industrial effluent extracts previously analysed for genotoxicity using the SOS Chromotest. Predictions are consistent with published mutagenic potency values for similar industrial waste materials. A consistent relationship was also identified between genotoxic potency and carcinogenic potency for 56 substances. Analysis of covariance revealed that the effect of SOS genotoxic potency covaries with experimental animal and route of exposure. The correlation between genotoxicity and carcinogenicity was fairly weak (maximum r value = 0.54). Previous studies revealed similar strength of association between Ames mutagenicity and carcinogenicity. The predicted carcinogenic potency of a previously tested, highly genotoxic industrial effluent extract was low compared to pure substances included in the dataset.

INTRODUCTION

It has been clearly established that a wide variety of xenobiotic substances pose a health hazard to humans and other biota. The goal of environmental toxicology is to identify these substances in order to regulate their production and release. While true hazard assessment is complicated by inadequate knowledge about emission rates, post-emission behaviour and *in situ* effects, environmental monitoring can identify sources of potential hazard. The efficacy of environmental monitoring requires that large numbers of complex samples be processed in a relatively short period of time (Lewtas, 1991). This requirement imposes the use of rapid, sensitive and inexpensive bioassays for (geno)toxicity assessment (Lewtas, 1991). Recent technological advances have made microbioassays increasingly popular for environmental monitoring and (geno)toxicity assessment (Blaise, 1991). In addition to meeting the above criteria, microbioassays have a conveniently small sample requirement (Lewtas, 1991; Blaise, 1991). However, these rapid, sensitive and convenient assays are not without drawbacks. While microbioassays facilitate rapid testing of large numbers of samples, it is often difficult to interpret the results obtained in the broader context of human or animal health.

Interpreting microbioassay results in the context of human health and safety is a dilemma that is familiar to genetic toxicologists. Microbioassays are frequently used to assess genotoxicity, mutagenicity and clastogenicity. The results are often used to infer mutagenic and carcinogenic hazard posed to humans and other biota (Venitt *et al.*, 1986; Kier, 1988; Bartsch *et al.*, 1980; Tennant *et al.*, 1987; Rodríguez-Ariza *et al.*, 1994; Britvic and Kurelec, 1986; de Raat *et al.*, 1985). One of the recently developed microbioassays for genotoxicity assessment is the SOS Chromotest (Quillardet *et al.*, 1982; Quillardet and Hofnung, 1985). The assay relies on *Escherichia coli*'s ability to invoke the SOS regulon in response to DNA damaging agents. The assay has several advantages over more traditional methods: 1) survival of the tester strain is not required; 2) the results can be obtained in a single working day; 3) sample sterility is not usually required 4) the organism responds to a wide range of DNA damage scenarios (Elsperu, 1987; Houk and deMarini, 1988) and 5) the test can easily accept biological samples such as tissue extracts and biological fluids (histidine contamination of biological samples can effect *Ames* test performance (Sparks *et al.*, 1981; Parry, 1985)). Despite these advantages, the test is plagued with the same interpretation problems as other microbioassays. While the ultimate consequences of exposure to SOS genotoxins will require *in situ* monitoring of exposed organisms, it is immediately possible to construct a broad empirical framework within which SOS Chromotest results can be interpreted. To do this, we investigated the empirical

relationships between genotoxic potency and mutagenic potency (as measured with the *Salmonella* microsome assay) as well as between genotoxic potency and carcinogenic potency (as measured using standard, chronic animal bioassays).

While several researchers have investigated the correspondence between SOS Chromotest results and *Ames* test results (Quillardet *et al.*, 1985; Ohta *et al.*, 1984; Quillardet and Hofnung, 1993; Von der Hude *et al.*, 1988), few have attempted to determine the quantitative relationship between genotoxic potency and mutagenic potency across a wide range of substances. The recent review by Quillardet and Hofnung (1993) determined that the correspondence between the SOS Chromotest and *Ames* test results is very high (over 82%). They suggested that a quantitative, empirical examination of the relationship between genotoxic potency and mutagenic potency would prove useful and interesting. We have used data from the literature to investigate this quantitative relationship.

Determination of the relationship between genetic toxicity and carcinogenicity has a long and controversial history. Recent results indicate that 60-70% of genotoxic substances are carcinogenic to mammals (see Quillardet and Hofnung, 1993; Claxton *et al.*, 1988; Tennant *et al.*, 1987; Haseman and Clark, 1990; Zeiger *et al.*, 1990; McCann *et al.*, 1988; Dunkel *et al.*, 1985). Quillardet and Hofnung (1993) demonstrated that 64% of SOS genotoxins are Class 1 and Class 2 carcinogens (as classified by Lewis, 1991). Despite the controversy, relatively few researchers have attempted to determine the quantitative relationship between genotoxic or mutagenic potency and carcinogenic potency across a large number of substances. Prior to 1985 this was at least in part due to the lack of a standardized database of carcinogenic potency. Quillardet and Hofnung (1993) suggested that the recently compiled carcinogenic potency database (Gold *et al.*, 1984, 1986, 1987, 1990 and 1993) can now be used to quantitatively compare genotoxic potency with carcinogenic potency. We have used the database of Gold *et al.* to investigate this relationship.

MATERIALS AND METHODS

Data Collection-

Mutagenic, genotoxic and carcinogenic potency data were collected from the recent literature. Genotoxic potency (SOS Chromotest) data were collected from 80 published works and 2 unpublished works. Following the method of Quillardet and Hofnung (1985), potency values are expressed as SOSIP (SOS response inducing potency), which is the slope of the initial, linear portion of the concentration-response relationship. Data were collected for 370 pure substances and 8 complex mixtures. The number of potency values collected for a given substance ranged from 1 to 30. All potency values were standardized to an assay volume of 1 mL and expressed as SOS induction factor units per μg of substance per mL of assay media. Unless otherwise indicated, the assay volume was assumed to be 0.62 mL (Quillardet and Hofnung, 1985). Where potency values were not provided, ordinary least squares regression was used to calculate the slope of the initial, linear portion of the concentration-response relationship of SOS induction factor versus concentration in μg per mL. Data presented on figures were obtained using digital image analysis. All potency values, except two from Llagostera *et al.*, (1986), were measured using the standard SOS chromotest bacteria- *Escherichia coli* PQ37 (see Huisman and D'Ari, 1981 and Quillardet and Hofnung, 1985 for details of strain construction and genotype). Potency values for substances that require metabolic activation were measured in the presence of Aroclor 1254-induced rat liver S9. For each substance the following information was collected- genotoxic potency, maximum SOS induction factor obtained, the presence of a metabolic activation system and the assay method employed. The collected potency values were categorized according to the following scheme-

Questionable response- maximum SOS induction factor never exceeds 1.5;

Marginal response- maximum SOS induction factor never exceeds 2.0 and a concentration-response relationship is evident;

Positive response- maximum SOS induction factor exceeds 2.0 and a concentration-response relationship is evident.

Mutagenic potency values are based on published *Salmonella typhimurium*/mammalian microsome test results (Ames *et al.*, 1973a; Ames *et al.*, 1973b). Mutagenic potency values were collected from 128 published studies. The number of mutagenic potency values collected for a given substance tested with a given *Salmonella* strain ranged from 1 to 11. Following Ames *et al.*, 1975, mutagenic potency is defined as the slope of the initial, linear

portion of the concentration-response relationship. All potency values were standardized to an assay volume of 1 mL and expressed as *his* revertents per μg of substance per mL of assay media (top agar) (Waters *et al.*, 1987). Unless otherwise indicated, top agar volume was assumed to be 2.2mL without S9 and 2.7mL with S9 (Maron and Ames, 1983). Where potency was not provided, ordinary least squares regression was used to calculate the slope of the initial linear portion of the concentration-response relationship of net *his* revertents versus concentration in μg per mL. Data presented on figures were obtained using digital image analysis. Mutagenicity was determined using 9 strains of *Salmonella typhimurium*; TA98, TA97, TA100, TA102, TA104, TA1530, TA1535, TA1538 and TA1537 (see Maron and Ames, 1983 and Claxton *et al.*, 1987 for description of tester strains). All potency values for substances that require metabolic activation were measured in the presence of Aroclor 1254-induced rat liver S9 (except metronidazole/mouse S9 - see Appendix 4). For each substance the following information was collected- mutagenic potency, ratio of the maximum number of revertents observed to background, *Salmonella* strain employed, the presence of a metabolic activation system and the assay method employed. The results were categorized according to the following scheme-

Questionable response- maximum number of revertents never exceeds 1.5 times background;

Marginal response- maximum number of revertents never exceeds 2.0 times background and a concentration-response relationship is evident;

Positive response- maximum number of revertents exceeds 2.0 times background and a concentration-response relationship is evident.

Only marginal and positive genotoxic and mutagenic potency values were used in the final data analyses. Mean mutagenic and genotoxic potency values for all substances used in the data analyses are provided in Appendix 4.

Carcinogenic potency data were obtained from the Carcinogenic Potency Database (CPDB) (Gold *et al.*, 1984; Gold *et al.*, 1986; Gold *et al.*, 1987; Gold *et al.*, 1990; Gold *et al.*, 1993). Briefly, the CPDB compiled the results of 4487 long-term, chronic experiments on 1136 chemicals. All data in the CPDB meet a set of fairly strict entry criteria. For example, the substance must have been tested alone, the study must have included a control group, the length of the experiment must be at least one year for rodents with dosing occurring for a minimum of six months (Gold *et al.*, 1991). Data were collected for 56 carcinogens that are capable of inducing an SOS response in *Escherichia coli* PQ37. Carcinogenic potency values are expressed as TD₅₀, a parameter that has been fully

described by Peto *et al.* (1984). Essentially, it is the dose of substance (in mg per kg body weight per day) that reduces the probability of remaining tumour-free at the end of the experiment (a standard life-time) by 50%. If the control animals do not develop any tumours, the TD₅₀ is simply the chronic dose that induces tumours in half the experimental animals. Potency values were collected only when a significant, positive response was obtained. A significant, positive response occurs when the slope of the dose-response relationship is significantly different from zero (Gold *et al.*, 1991). For each carcinogen, carcinogenic potency, experimental animal and route of exposure were noted. Due to data limitations no attention was paid to target organ, tumour histopathology, animal sex or animal strain. The number of potency values collected for a given substance/animal/exposure route combination ranged from 1 to 47. Geometric mean carcinogenic potency values for each of the 56 substances used in the final data analyses are summarized in Appendix 4.

Data Analysis

Relationships between mean SOS genotoxic potency and 1) mean *Ames* mutagenic potency as well as 2) mean carcinogenic potency were investigated using ordinary, least squares regression and analysis of covariance (ANCOVA) employing SAS version 6.08 (SAS institute, 1988) and SYSTAT (Wilkinson, 1987). The data were fitted to the model $Y_i = \beta_0 + \beta_1(X_1) + \beta_2(X_2) + \dots + \beta_k(X_k) + \epsilon$, where Y_i is mutagenic potency in *his* revertents per μg substance per assay mL or carcinogenic potency (as TD₅₀) in mg per kg body weight per day, X_1 is SOS genotoxic potency in induction factor units per μg substance per assay mL and X_2 through X_k are variables that indicate *Salmonella* strain, test animal and route of exposure. The stochastic error term ϵ was assumed to be independent and normally distributed with a mean of zero and variance σ^2 . Normality of the error term from each model was assessed using the Komogorov-Smirnov test (Zar, 1984) and visual inspection of a normal probability plot (Wilkinson, 1987). The data were log transformed to equalize the variance over the range of observations and meet the normality assumptions of least squares regression analysis (Ricker, 1973). To account for the possibility of underestimating the regression coefficients (slope(s)) when there is error in the independent variable, a model II or geometric mean slope was calculated as recommended by Ricker (1973). To compensate for the inherent bias in log transformed equations, correction factors were calculated ($\text{CF} = \exp(1.1513 \times \text{MSE})$ where MSE is the mean square error of the regression). Predictions made using regression models based on log transformed variables must be multiplied by the correction factor following conversion back to an arithmetic scale (Neyman and Scott, 1960).

The following abbreviations are used in the text: SOSIP = SOS response inducing potency in SOS induction factor units per μg of substance per assay mL, IF = SOS induction factor in multiples of the control (solvent blank), MUTPOT = *Ames* test mutagenic potency in histidine revertents per μg substance per assay (top agar) mL, CF = correction factor, Rev = *his* revertents, Gav = gavage exposure, Eat = exposure through diet, Wat = exposure through water, Inh = exposure through inhalation, Ipi = exposure through intraperitoneal injection, Ivi = exposure through intravenous injection, Mix = multiple routes of exposure, Ori = gavage exposure as preweanling followed by exposure through diet.

RESULTS

Mean, minimum and maximum values of SOS genotoxic potency, *Ames* mutagenic potency and carcinogenic potency are summarized in Table 1. Genotoxic potency and mutagenic potency values are divided into direct acting substances and substances that require S9 metabolic activation. Mean mutagenic potency values are provided for each tester strain. Extreme values are worth noting. The highest mutagenic and genotoxic potency values were obtained for a 2-nitronaphthofuran derivative (R7371) assayed using *Salmonella* strain TA100 (Arnaise *et al.*, 1986). The lowest potency values were obtained for a urine extract tested using the frameshift mutagen strain TA98 (Venier *et al.*, 1989). In general, the highest genotoxic and mutagenic potency values were obtained for nitroarenes such as 1,8-dinitropyrene and several 2-nitronaphthofuran derivatives. The lowest potency values were obtained for extracts of complex mixtures such as smoker's urine, urine from patients receiving coal tar treatment for psoriasis (Venier *et al.*, 1989) and Portuguese red wine (Rueff *et al.*, 1986). Carcinogenic potency values are summarized both by animal and route of exposure. The highest TD₅₀ value (i.e. low potency carcinogen) was obtained for quercetin tested in mice via diet exposure. The lowest value was obtained for mitomycin C tested in rats by intraperitoneal injection.

Using the entire data set of positive and marginal direct acting genotoxins, analysis of covariance revealed that mutagenic potency is determined by SOS genotoxic potency and *Salmonella* tester strain employed. Although strain has a significant effect on mutagenic potency, post-hoc contrasts of the regression coefficients revealed that two strain groups, rather than eight, is sufficient. The relationship between direct-acting mutagenic potency and SOS genotoxic potency and strain group is:

$$\begin{array}{l} \text{Log Mutagenic Potency} \\ \text{(his revertents per } \mu\text{g per mL)} \end{array} = \begin{array}{l} \boxed{\begin{array}{l} 2.36 \text{ TA100, TA102, TA104, TA97, TA1538} \\ 1.39 \text{ TA1537, TA1535, TA98} \end{array}} \begin{array}{l} \text{A} \\ \text{B} \end{array} + 0.80 * (\text{Log SOSIP}) \quad (1)$$

$r^2 = 0.71$, $n = 465$, $F \text{ Ratio} = 551.8$, $p < 0.0001$. Model II coefficient for Log SOSIP = 0.95. Number of substances = 256. Intercept values accompanied by the same letter are not significantly different at $\alpha = 0.05$. Higher level effects were not significant at $\alpha = 0.05$. Additional regression statistics are provided in Table 2.

Table 1. Summary statistics. Mean, minimum and maximum values of *Ames* mutagenic potency (*his* revertants per μg per mL), SOS Chromotest genotoxic potency (IF per μg per mL) and animal carcinogenic potency (TD_{50} in mg per kg body weight per day). Mutagenic potency data are broken down according to *Salmonella typhimurium* strain employed. Carcinogenic potency data are broken down both by experimental animal and route of exposure.

Genotoxic and Mutagenic Potency Data				
1) Without S9 Metabolic Activation				
Mutagenic Potency				
	N	Minimum	Maximum	Mean
TA100	200	5.10E-02	4.94E+07	3.74E+05
TA102	21	1.55E+00	7.29E+04	7.55E+03
TA104	20	1.39E-01	2.33E+06	1.38E+05
TA1535	30	8.08E-02	4.78E+03	2.23E+02
TA1537	24	1.53E-03	4.36E+05	3.70E+04
TA1538	23	1.82E+00	1.17E+05	1.66E+04
TA97	31	1.16E-01	4.36E+05	3.26E+04
TA98	118	3.84E-05	2.84E+06	8.74E+04
Genotoxic Potency				
SOSIP (PQ37)	467	6.27E-07	2.14E+05	4.47E+03
2) With S9 Metabolic Activation				
Mutagenic Potency				
	N	Minimum	Maximum	Mean
TA100	102	2.67E-01	5.02E+05	1.67E+04
TA102	3	4.89E+00	6.88E+02	2.55E+02
TA1530	3	1.40E-00	2.56E+00	1.87E+00
TA1535	10	1.38E-01	1.62E+02	3.99E+01
TA1537	13	2.56E-01	2.23E+02	3.19E+01
TA1538	16	1.08E+00	2.13E+04	2.37E+03
TA97	15	4.03E+00	1.57E+03	2.80E+02
TA98	52	5.39E-06	3.39E+05	1.85E+04
Genotoxic Potency				
SOSIP (PQ37)	214	2.64E-08	3.61E+03	2.78E+01

Table 1. continued.....

Carcinogenic Potency Data (TD ₅₀)				
	N	Minimum	Maximum	Mean
By Animal				
Hamster	4	5.61E+00	7.81E+01	2.90E+01
Mouse	43	4.25E-03	4.24E+03	2.08E+02
Primate	6	3.11E-02	1.92E+01	5.02E+00
Rat	47	1.66E-03	5.96E+02	5.69E+01
By Route of Exposure				
Eat	36	8.46E-03	4.24E+03	2.82E+02
Gav	23	1.68E-01	2.61E+02	3.59E+01
Inh	9	4.77E-02	1.81E+02	3.27E+01
Ipj	7	1.66E-03	4.96E+00	1.48E+00
Ivj	1	2.07E-02	2.07E-02	2.07E-02
Mix	2	7.03E-02	5.74E+00	2.91E+00
Orl	4	3.96E+00	1.17E+02	5.15E+01
Wat	18	3.98E-02	7.81E+01	1.52E+01

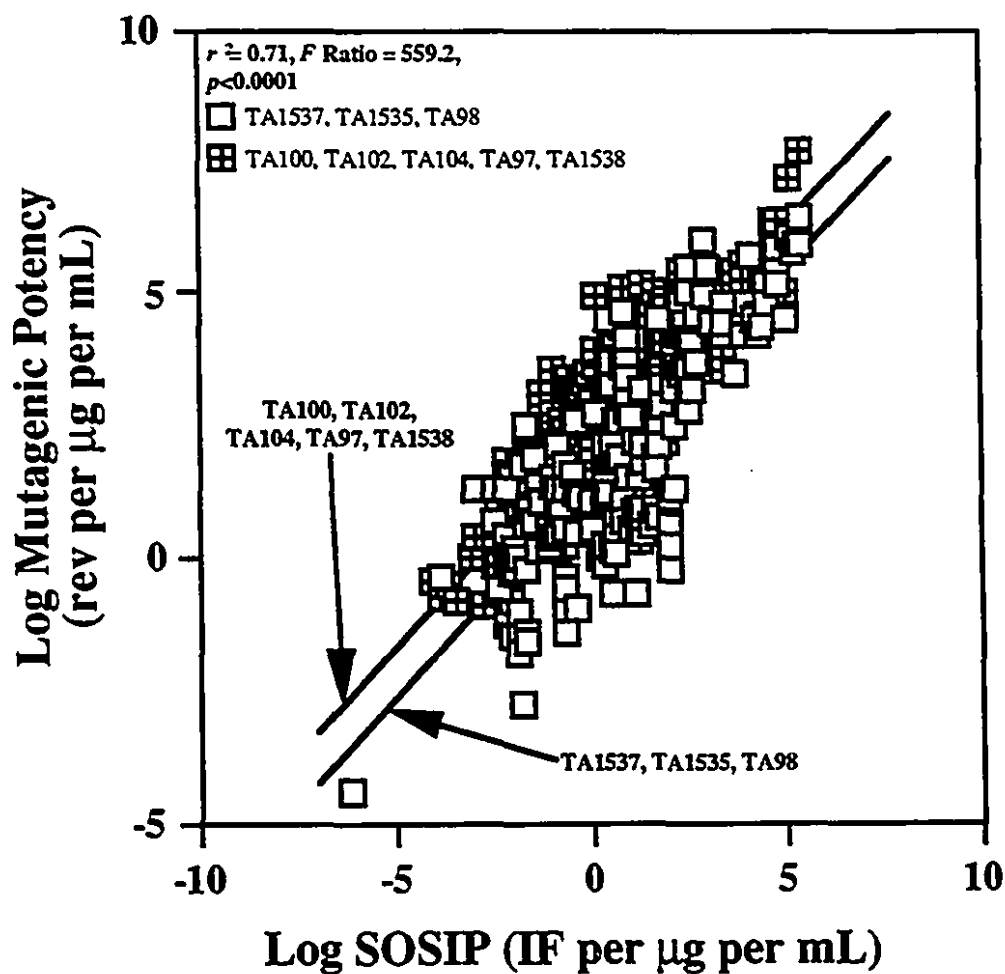
Table 2. Miscellaneous regression statistics. CF is a correction factor required for conversion of model predictions to an arithmetic scale. Predictions converted to arithmetic values must be multiplied by the correction factor to account for the inherent bias in log transformed equations. Mean X (i.e. Log SOSIP), ΣX^2 and MSE (the mean square error of the model) are required to calculate confidence limits on individual model predictions. $\Sigma(\text{PRESS})$ is the sum of the predicted residual error sum of squares (Allen, 1971). Calculation of the PRESS values involve omitting each observation in turn from the data set, fitting the model to the remaining observations, predicting the value of the omitted observation and comparing the prediction with the observed value. The sum of the squared differences between the omitted observations and their predicted values provides the $\Sigma(\text{PRESS})$. When this value is minimized (for a given dataset), one selects the model with the greatest predictive value (Aitkin *et al.*, 1981).

Model	CF	Mean X	ΣX^2	$\Sigma(\text{PRESS})$	MSE
(1)	3.50	0.19	1701.31	558.97	1.09
(2)	2.62	0.19	1701.31	368.72	0.84
(3)	5.13	-0.43	545.21	310.41	1.42
(4)	3.03	-0.43	545.21	197.48	0.96
(5)	3.94	0.19	1701.31	304.02	1.19
(6)	3.07	0.43	545.21	117.84	0.97
(7)	4.56	-0.74	358.84	150.48	1.32
(8)	5.70	-0.79	129.18	82.03	1.51

The effect of SOS genotoxicity and *Salmonella* strain on the mutagenic potency of direct-acting substances is illustrated in Figure 1. It is interesting to note that both the slope and intercept values are significantly different and slightly smaller than the values published by DeMéo *et al.* (1992) in their study of the genotoxicity and mutagenicity (on TA100) of nitroimidazoles.

Analysis of the residuals (observed Y - predicted Y) from model (1) revealed some interesting patterns. For example, 75% of the positive residuals > 2.0 are nitroarenes such as dinitropyrenes. Cluster analysis was used to further investigate patterns in the residuals. Distances between clusters were assigned using the centroid method, where distance is defined as the squared euclidean distance between the cluster centroids (SAS Institute,

Figure 1. The relationship between *Ames* mutagenic potency (*his* revertants per μg substance per assay mL) and SOS genotoxic potency (IF per μg per assay mL) for direct acting substances. The fitted lines represent the ANCOVA model (model (1)) discussed in the text. *Salmonella* strain employed covaries with the effect of SOS genotoxicity. The fitted lines represent the two groups of *Salmonella* strains revealed by post-hoc contrasts of regression coefficients for each strain (Group 1 = TA100, TA102, TA104, TA97 and TA1538; Group 2 = TA1537, TA1535 and TA98). The two intercepts are significantly different at $\alpha=0.05$. Higher level effects ie. interactions between log SOSIP and strain group were not significant at $\alpha=0.05$.



1988). The results obtained indicate that in many cases the above model dramatically underpredicts the mutagenicity of nitroarenes on TA98. The cluster analysis identified 12 nitroarenes that on average are 2.0 orders of magnitude more potent on TA98 than model (1) predicts. In addition, the model overpredicts the mutagenic potency of several compounds tested on TA1535, TA1537 and TA1538. The cluster analysis identified 9 substances that on average are 2 orders of magnitude less potent than model (1) would predict. These values were removed from the data set (5 substances completely deleted) and the above model reconstructed. The new model, with slightly changed coefficients and r^2 is:

$$\begin{array}{l} \text{Log Mutagenic Potency} \\ \text{(his revertants per } \mu\text{g per mL)} \end{array} = \begin{array}{|l|} \hline 2.39 \text{ TA100, TA102, TA104, TA97, TA1538} \\ 1.36 \text{ TA1537, TA1535, TA98} \\ \hline \end{array} \begin{array}{|c|} \hline \text{A} \\ \text{B} \\ \hline \end{array} + 0.80 * (\text{Log SOSIP}) \quad (2)$$

$r^2 = 0.76$, $n = 437$, $F \text{ Ratio} = 701.8$, $p < 0.0001$. Model II coefficient for Log SOSIP = 0.92. Number of substances = 251. Intercept values accompanied by the same letter are not significantly different at $\alpha = 0.05$. Higher level effects were not significant at $\alpha = 0.05$. Additional regression statistics are provided in Table 2.

In a similar manner, analysis of covariance revealed a significant effect of SOS genotoxicity and *Salmonella* strain on the mutagenic potency of substances that require S9 metabolic activation. However, in this case a significant interaction between SOS genotoxic potency and *Salmonella* strain was identified. Post-hoc contrasts of the regression coefficients revealed that three strain groups is sufficient. The relationship between S9 activated mutagenic potency and SOS genotoxic potency and strain group is:

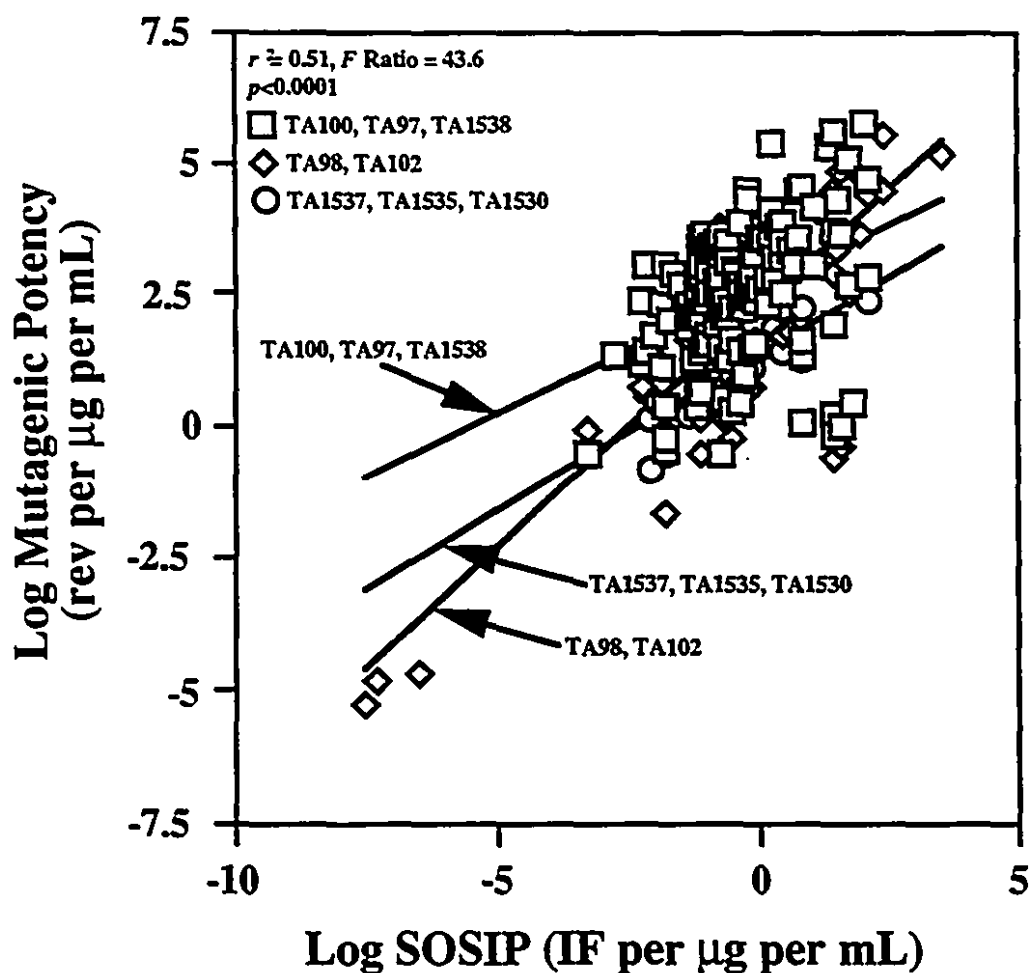
$$\begin{aligned}
 \text{Log Mutagenic Potency} &= \begin{bmatrix} 2.63 & \text{TA100, TA97, TA1538} & \text{A} \\ 2.22 & \text{TA98, TA102} & \text{B} \\ 1.31 & \text{TA1537, TA1535, TA1530} & \text{C} \end{bmatrix} \\
 &+ \begin{bmatrix} 0.48 & \text{TA100, TA97, TA1538} & \text{A} \\ 0.89 & \text{TA98, TA102} & \text{B C} \\ 0.59 & \text{TA1537, TA1535, TA1530} & \text{A C} \end{bmatrix} * (\text{Log SOSIP}) \quad (3)
 \end{aligned}$$

$r^2 = 0.51$, $n = 214$, F Ratio = 43.6, $p < 0.0001$. Model II coefficients for Log SOSIP are 0.67, 1.24 and 0.82. Number of substances = 122. Regression coefficients accompanied by the same letter are not significantly different at $\alpha = 0.05$. Higher level effects were not significant at $\alpha = 0.05$. Additional regression statistics are provided in Table 2.

The intercept values obtained are similar to those in models (1) and (2). The slope terms are all slightly lower than those in models (1) and (2). Post-hoc contrasts of the SOSIP coefficients indicated that the effect of SOSIP on mutagenic potency is significantly larger when TA98 or TA102 are used to measure mutagenicity. These results are illustrated on Figure 2.

Analysis of the residuals from the above model again revealed some interesting patterns. All of the positive residuals > 2.0 are nitroarenes such as 2,7-dinitro-9-fluorenone. Again cluster analysis was used to further investigate patterns in the residuals. The results obtained again indicate that in several cases the above model dramatically underpredicts the mutagenicity of nitroarenes. The cluster analysis identified 5 nitroarenes that on average are 1.9 orders of magnitude more potent on TA98 than the above model predicts. In addition, the model overpredicts the mutagenic potency of several tranquilizing constituents of *Valerianaceae* roots (Von der Hude *et al.*, 1986). The cluster analysis revealed that on average the 4 *Valerianaceae* root constituents valtrate, dihydrovaltrate, baldrrinal and homobaldrrinal are 3.6 orders of magnitude less potent than the model would predict. These data were removed from the data set (4 substances completely deleted) and

Figure 2. The relationship between *Ames* mutagenic potency (*his* revertants per μg substance per assay mL) and SOS genotoxic potency (IF per μg per assay mL) for substances that require S9 metabolic activation. The fitted lines represent the ANCOVA model (model (3)) discussed in the text. *Salmonella* strain employed covaries with the effect of SOS genotoxicity. In addition, an interaction between Log SOSIP and *Salmonella* strain was detected. The fitted lines indicate the slopes and intercept for the three groups of *Salmonella* strains revealed by post-hoc contrasts of regression coefficients (Group 1 = TA100, TA97 and TA1538; Group 2 = TA98 and TA102; Group 3 = TA1537, TA1535 and TA1530). The three intercepts are significantly different at $\alpha=0.05$. The slope for TA1537, TA1535 and TA1530 (Group 3) is not significantly different from the slope for TA100, TA97 and TA1538 (Group 1).



the above model reconstructed. The new model, with slightly changed coefficients and r^2 , is:

$$\begin{aligned} \text{Log Mutagenic Potency} \\ (\text{his revertents per } \mu\text{g per mL}) &= \begin{bmatrix} 2.80 & \text{TA100, TA97, TA1538} & \text{A} \\ 2.23 & \text{TA98, TA102} & \text{B} \\ 1.31 & \text{TA1537, TA1535, TA1530} & \text{C} \end{bmatrix} \\ &+ \begin{bmatrix} 0.66 & \text{TA100, TA97, TA1538} & \text{A C} \\ 0.96 & \text{TA98, TA102} & \text{B C} \\ 0.59 & \text{TA1537, TA1535, TA1530} & \text{A} \end{bmatrix} * (\text{Log SOSIP}) \quad (4) \end{aligned}$$

$r^2 = 0.66$, $n = 202$, F Ratio = 75.7, $p < 0.0001$. Model II coefficients for Log SOSIP are 0.81, 1.20 and 0.72. Number of substances = 118. Regression coefficients accompanied by the same letter are not significantly different at $\alpha = 0.05$. Higher level effects were not significant at $\alpha = 0.05$. Additional regression statistics are provided in Table 2.

Cluster analyses of the residuals from models (1) and (3) also revealed that the models can make better predictions about the mutagenicity of complex mixtures as compared with pure substances. For example, the mean absolute value of the residuals for the 7 complex mixture samples included in the model (1) data is 0.56, while the root mean square error of the model is 1.04. With respect to model (3), the mean absolute value of the residuals for 9 complex mixture samples is 0.73, while the root mean square error is 1.42.

From a purely predictive point of view, it is useful to construct empirical models that relate maximum mutagenic potency (regardless of strain) to SOS genotoxic potency. Using data for 255 direct acting substances, the relationship between maximum mutagenic potency and SOS genotoxic potency is:

$$\begin{aligned} \text{Log Max. Mutagenic Potency} \\ (\text{his revertents per } \mu\text{g per mL}) &= 2.28 + 0.78 * (\text{Log SOSIP}) \quad (5) \end{aligned}$$

$r^2 = 0.69$, $n = 254$, F Ratio = 551.7, $p < 0.0001$. Model II coefficient for Log SOSIP = 0.94. Additional regression statistics are provided in Table 2.

Using data for 118 S9 activated substances, the relationship between maximum mutagenic potency and SOS genotoxic potency is:

$$\text{Log Max. Mutagenic Potency} \quad = \quad 3.64 \quad + 0.94 * (\text{Log SOSIP}) \quad (6)$$

(his revertents per μg per mL)

$r^2 = 0.71$, $n = 118$, F Ratio = 282.3, $p < 0.0001$. Model II coefficient for Log SOSIP = 1.11. Additional regression statistics are provided in Table 2.

The empirical models described above can be used to predict the *Ames* mutagenic potency of a substance that has been tested on the SOS Chromotest. Such predictions permit the use of the SOS Chromotest and the subsequent comparison of the results obtained to the large body of *Ames* test results available in the literature (see Houk, 1992 for complex industrial wastes and Dunkel *et al.*, 1985; Dunkel *et al.*, 1984; Haworth *et al.*, 1983 among others for pure substances). Models (1) and (5) were tested for their ability to predict the *Ames* mutagenic potency of several industrial effluent extracts that were examined for genotoxicity using the SOS Chromotest. Predictions are summarized in Table 3. Although simultaneous mutagenic potency results are not available, published *Ames* test data for several industrial wastes are provided for comparison (Houk, 1992). The results indicate that the predicted potency values are fairly similar to mean potency values based on data presented in the literature.

Initial attempts to correlate carcinogenic potency and SOS genotoxic potency were not entirely successful. When all available data were included, the regression coefficient was not significantly different from zero ($p=0.064$). Analysis of the residuals indicated that four compounds are far less carcinogenic (mean residual = 2.5) than a model based solely on genotoxic potency would predict. When these compounds (captan, captafol, furylfuramide and nitrofurantoin) were removed from the data set, a significant correlation was obtained (Pearson $r = 0.44$, $p < 0.0001$, number of substances = 52). Analysis of covariance (captan, captafol, furylfuramide and nitrofurantoin included) revealed that carcinogenic potency is also determined by the experimental animal used and the route of exposure. Post-hoc contrasts of regression coefficients revealed the following model with two experimental animal groups and three exposure groups:

Table 3. Predicted *Ames* mutagenic potency (in *his* revertents per mg extractable residue per mL) of industrial effluent extracts.

Sample Type	Mutagenic Potency Values Predicted From SOS Genotoxicity Results*			Ames Mutagenic Potency Values From Houk (1992) [†]		
	Model (1) Prediction - Group 1 Strains	Model (1) Prediction - Group 2 Strains	Model (5) Prediction	Max. Value	Min. Value	Mean Value
1) Organic chemical production facilities	1.91E+04	4.95E+03	1.96E+04	5.9E+04	3.3E+02	1.9E+04
2) Metal refining and foundry facilities	3.08E+03	3.30E+02	3.31E+03	7.3E+03	5.5E+02	3.9E+03
3) Petroleum refining/ petro-chemical facilities	6.58E+03	7.06E+02	6.95E+03	5.9E+03	2.6E+02	1.9E+03
4) Pulp and paper production facilities	1.27E+03	1.36E+02	1.40E+03	1.1E+04	3.9E+02	5.0E+03

*Industrial effluent genotoxicity data are from White *et al* (1995). Three dichloromethane extracts were prepared from each sample- suspended particulate extract, aqueous acidic extract and aqueous alkaline extract. Results presented are mean SOS genotoxic potency values (in IF per mg residue per assay mL) for the most potent fraction. Sample size for each category ranged from 4 to 6. The two model (1) predictions represent the different *Salmonella* strain groups revealed by the analysis of covariance (Group 1 = TA100, TA102, TA104, TA97 and TA1538; Group 2 = TA1537, TA1535 and TA98). The model (5) prediction is the maximum mutagenic potency, irrespective of strain employed.

[†]Means are based on 2 to 8 observations. Assuming an assay volume 2.2mL, potency values were converted to *his* revertents per mg residue per mL.

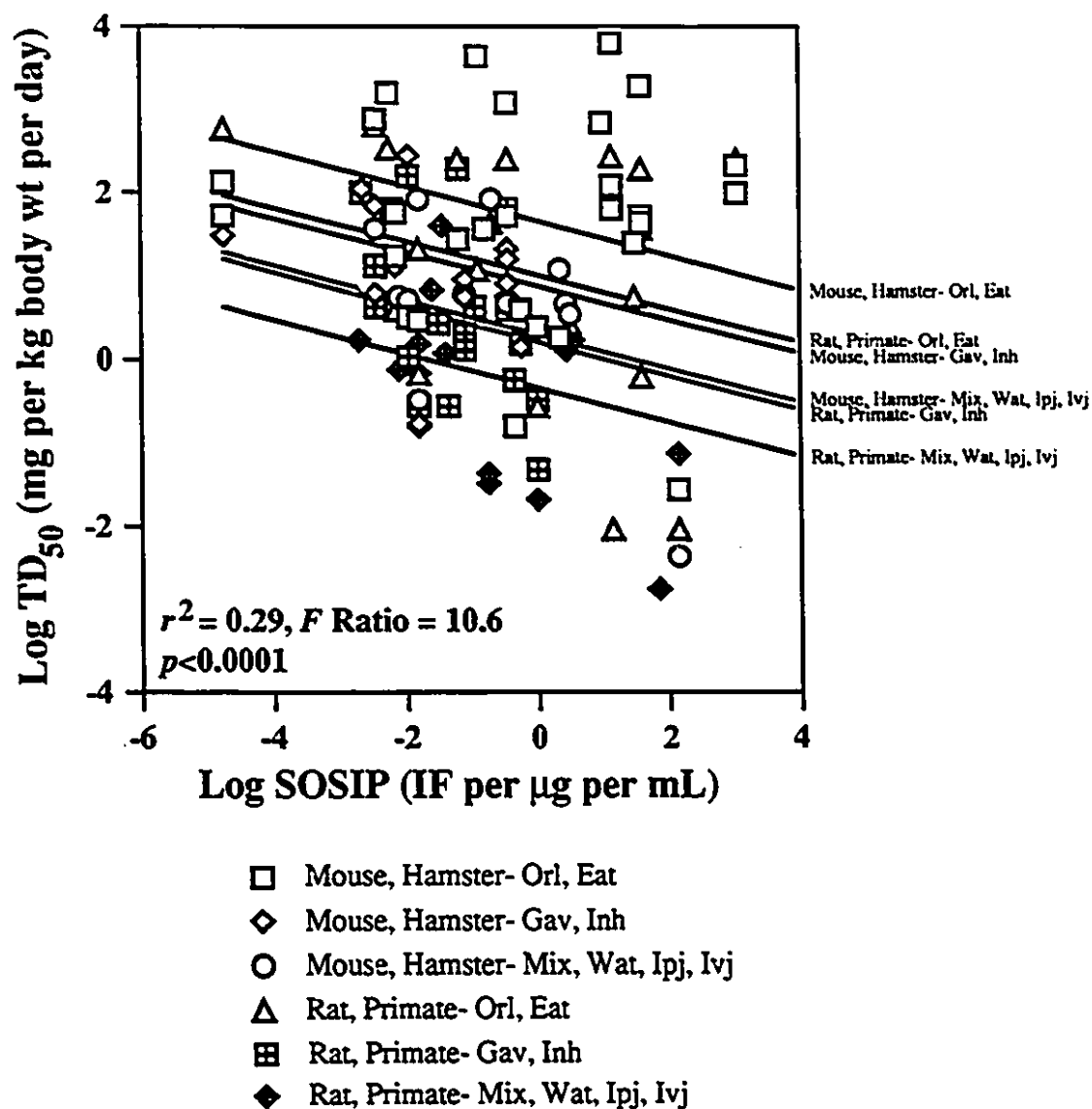
$$\text{Log Carcinogenic Potency} \\ (\text{TD}_{50} \text{ in mg per kg body wt per day}) =$$

+ 1.64 Mouse, Hamster- Orl, Eat + 0.99 Rat, Primate Orl, Eat + 0.87 Mouse, Hamster- Gav, Inh + 0.29 Mouse, Hamster- Mix, Ipj, Ivj, Wat + 0.22 Rat, Primate- Gav, Inh - 0.36 Rat, Primate- Mix, Ipj, Ivj, Wat	A A C A C D B C D B C D B	$- 0.20 * (\text{Log SOSIP})$	(7)

$r^2 = 0.29$, $n = 109$, F Ratio = 10.6, $p < 0.0001$. Model II coefficient for Log SOSIP = -0.38. Number of substances = 56. Intercept values accompanied by the same letter are not significantly different at $\alpha = 0.05$. Higher level effects were not significant at $\alpha = 0.05$. Additional regression statistics are provided in Table 2.

The negative slope indicates that potent genotoxins have low TD_{50} 's i.e. are potent carcinogens. Differences in intercept indicate that rats and primates are more sensitive test animals and exposure through water, intraperitoneal or intravenous injection is more effective than other routes. These results are illustrated on Figure 3. Model (7) and Figure 3 indicate that the effect of genotoxic potency on carcinogenic potency is weak. Although the slope is significantly different from zero, it is quite shallow. It should be noted that the slope value is decreased by substances that are highly genotoxic and only mildly carcinogenic. Such observations frequently have high leverage values that suggest undue influence on the regression coefficients (Velleman and Welsch, 1981). For example, the removal of several observations with high genotoxic potency and low carcinogenic potency (furylfuramide- rat, primate via orl, eat; nitrofurantoin- mouse, hamster via orl, eat and captan- mouse, hamster via orl, eat) decreases the slope by 30% from -0.20 to -0.26. Some published analyses of the relationship between carcinogenic potency and mutagenic potency (e.g. McCann *et al.*, 1988; Piegorsch and Hoel, 1988) used the most potent TD_{50} value available for a given target site. We also examined the relationship between the maximum, significant TD_{50} value recorded for each compound and genotoxic potency. The correlation is only significant after data for captan, captafol, furylfuramide and nitrofurantoin are removed.

Figure 3. The relationship between carcinogenic potency (TD_{50} in mg per kg body weight per day) and SOS genotoxic potency (IF per μg per assay mL). The ANCOVA model described in the text (model (7)) indicates that the effect of SOS genotoxicity covaries with experimental animal and route of exposure. Post-hoc contrasts of regression coefficients revealed a model with six animal/route combinations. Higher level effects, ie. interactions between log SOSIP and animal/route group were not significant at $\alpha=0.05$.



The relationship identified is:

$$\text{Log Max. Carcinogenic Potency} \\ (\text{TD}_{50} \text{ in mg per kg body wt per day}) = 1.00 - 0.42 * (\text{Log SOSIP}) \quad (8)$$

$r^2 = 0.18$, $n = 52$, $F \text{ Ratio} = 11.2$, $p < 0.0015$. Model II coefficient for Log SOSIP = -0.97. Additional regression statistics are provided in Table 2.

Table 4 shows a comparison of the correlations between genotoxic potency and carcinogenic potency presented in this study and similar correlations between Ames mutagenic potency and carcinogenic potency presented in the literature. Literature values presented were obtained from studies that examined a wide variety of substances. With the exception of the study by Meselson and Russel (1977), published simple correlation coefficients are in the 0.24 to 0.44 range. Similar strength of association between carcinogenic potency and genotoxic potency was revealed in this study. However, when experimental animal and route of exposure are included as additional variables, the correlation coefficient determined in this study ($r = 0.54$) is greater than the published values summarized in Table 4.

Table 4. Comparison of several quantitative correlations between genotoxic potency or *Ames* mutagenic potency and carcinogenic potency. Correlations between SOS genotoxic potency and carcinogenic potency were obtained in this study (models 7 and 8). Correlations between mutagenic potency and carcinogenic potency were obtained from the literature. *p* values are based on critical values for the simple product-moment correlation coefficient with (n-2) degrees of freedom. NS = not significant at $p < 0.05$.

Variables Correlated	Simple Correlation Coefficient (<i>r</i>) [†]	Number of Substances Examined	Reference
Carcinogenic potency vs. <i>Ames</i> mutagenic potency	0.24 to 0.41 ($p < 0.05$ to $p < 0.001$)	77 to 89	McCann <i>et al.</i> , 1988.
Carcinogenic potency vs. <i>Ames</i> mutagenic potency	0.92 ($p < 0.001$)	9	Meselson and Russel, 1977.
Carcinogenic potency vs. <i>Ames</i> mutagenic potency	0.36 ^{NS}	14	Meselson and Russel, 1977.
Carcinogenic potency vs. <i>Ames</i> mutagenic potency	0.39 ($p < 0.001$)	88	Parodi <i>et al.</i> , 1982.
Carcinogenic potency vs. <i>Ames</i> mutagenic potency	0.44 ($p < 0.001$)	28	Piegorsch and Hoel, 1988.
Carcinogenic potency vs. <i>Ames</i> mutagenic potency	0.34 ($p < 0.01$)	59	Parodi <i>et al.</i> , 1983.
Mean carcinogenic potency vs. SOS Genotoxic potency	0.44 ($p < 0.002$)	52	This study.
Max. carcinogenic potency vs. SOS Genotoxic potency	0.43 ($p < 0.002$)	52	This study.
Mean carcinogenic potency vs. SOS Genotoxic potency, animal and route of exposure	0.54 ($p < 0.001$)	56	This study.

[†]Pearson product-moment correlation coefficients (Zar, 1984).

DISCUSSION

The goal of this work was to construct a broad empirical framework within which SOS chromotest results can be interpreted. To do this we investigated the quantitative relationships between genotoxic potency and both mutagenic potency and carcinogenic potency. This was done using linear regression and analysis of covariance on data collected from the recent literature. Before discussing the results it is appropriate to discuss some of the limitations of the data. Only 2% of the substances in the dataset are complex mixtures. Most of the *Salmonella*/microsome assay data (almost 70%) were determined using tester strains TA98 or TA100. This is not surprising since these strain have emerged as the most popular and efficacious strains for testing both pure compounds (Zeiger *et al.*, 1985) and complex mixtures (Houk, 1992). In addition, although the dataset contains mutagenic potency values measured using 9 different strains of *Salmonella typhimurium*, the dataset is not balanced. Thus, for each compound in the dataset, potency values were only available for a subset of the possible *Salmonella* strains. This fact imposes restrictions on the interpretation of the results. Although post-hoc contrasts of regression coefficients revealed different strain groups, it is difficult to determine whether these differences reflect real differences in the sensitivities of individual *Salmonella* strains or are simply empirical consequences that reflect the unbalanced nature of the dataset. Most of the carcinogenic potency measurements (90%) were obtained using mice and rats. In addition, in most of the carcinogenic potency assays (77%) the animal was exposed using diet, gavage or water exposure.

Using data on 257 substances we established a clear, positive relationship between genotoxic potency and Ames test mutagenic potency for direct acting substances. Mutagenic potency, as measured using the Ames test is empirically determined by genotoxic potency and the strain of *Salmonella typhimurium* employed. The results, presented in Figure 1 and models (1) and (2), indicate that over 70% of the variation in mutagenic potency can be explained by SOS genotoxic potency and *Salmonella* strain. The remaining unexplained variation can likely be attributed to wide variety of methodological variations that are known to effect potency measurements. Among the factors thought to effect potency are inoculum size, mutagen solvent, histidine concentration and preincubation time (Maron and Ames, 1983; McCann *et al.*, 1984; Gatehouse, 1987). The coefficient of SOSIP in model (1) is significantly less than 1.0. Therefore, the relationship between mutagenic potency and genotoxic potency is not linear. The large intercept values (1.39 to 2.36) indicate that mutagenic potencies are generally one to two orders of magnitude greater than the corresponding SOS genotoxic potencies. The results further

indicate that there is a significant difference (one order of magnitude) between the potency measured with Group 1 strains (TA100, TA102, TA104, TA97 and TA1538) and potency measured using Group 2 strains (TA1537, TA1535 and TA98). Despite the aforementioned unbalanced nature of the dataset, this strictly empirical result matches reasonable well with discussions of *Ames* strain potency encountered in the literature. Two of the three *less sensitive* Group 2 strains do not contain the R-factor plasmid (pKM101) that enhances error-prone (SOS response mediated) DNA repair and increases sensitivity for mutagen detection (Maron and Ames, 1983; Claxton *et al.*, 1987). Five of the six *more sensitive* Group 1 strains contain the R-factor plasmid. In addition, four of the five Group 1 strains are noted for their high degree of sensitivity. TA100, a strain used to detect base-pair substitution mutagens, is noted for its high sensitivity in mutagen detection relative to strains TA98, TA1535, TA1537 and TA1538 (Herbold, 1982; Zeiger *et al.*, 1985). TA97 has a *hot spot* of G-C pairs in the *hisD6610* mutation that make it highly sensitive to frameshift mutagens (Levin *et al.*, 1982a). TA102 and TA104 are used for the detection of oxidative mutagens that are poorly detected by the *standard* set of tester strains (Maron and Ames, 1983; Levin *et al.*, 1982b). The presence of TA1538, a strain that does not contain the R factor plasmid, in the Group 1 strains is likely due the high potency of compounds for which TA1538 data were available. Appendix 4 and Table 1 indicate that many of the substances for which TA1538 data were available have mutagenic potency values in the 10^4 - 10^5 revertents per μg per mL range.

The cluster analyses revealed that nitroarenes are far more mutagenic to TA98 than models (1) or (3) would predict. This may not be surprising since some nitroarenes (e.g. dinitropyrenes) have been called the most potent bacterial mutagens (Mersch-Sundermann *et al.*, 1992b; Mermelstein *et al.*, 1981). Genotoxic activity of these compounds is generally attributed to the substituted carbon *para* to the nitro group (Mersch-Sundermann *et al.*, 1992b). However, the result is not consistent with the strong correlation between SOS genotoxic potency and mutagenic potency for 13 nitroarenes assayed on TA98 reported by Mersch-Sundermann *et al.* (1991a). The high positive residuals of nitroarenes in model (1) may simply be caused by the extreme potency of the nitroarenes on TA98 (e.g. nitrofluorene data from McCoy *et al.*, 1981 and nitropyrene data from Mermelstein *et al.*, 1981) relative to the other substances for which TA98 data were available. This supposition is partially confirmed by the drop in the mean nitroarene residual from >2.0 to <0.8 , when maximum mutagenic potency is used as the dependant variable (model (5)). The residuals from model (5) revealed that 2,7-dinitro-9-fluorenone is the only nitroarene that is still 2 orders of magnitude more potent than the model would predict. It is interesting to note that model (1) also underpredicts the potency of organic extracts from diesel

particulate matter (0.8 orders of magnitude i.e. actual potency = 6 fold higher than prediction). This may not be surprising since the high potency of diesel particulate matter is often attributed to nitroarenes (Pedersen and Siak, 1981; Saleem *et al.*, 1984).

The cluster analysis also revealed several compounds that are far less potent on the non-R factor strains TA1535, TA1537 and TA1538 than model (1) would predict. Most of these compounds have mutagenic potencies on the non-R factor strains that are 1 to 2 orders of magnitude lower than those obtained with the matching R factor strains (Ames *et al.*, 1975; McCann *et al.*, 1975). For example, 4-nitroquinoline-*N*-oxide is almost 2 orders of magnitude more potent when the R factor plasmid is present (Levin *et al.*, 1982a; McCann *et al.*, 1975).

Using data on 122 substances we established a clear, positive relationship between genotoxic potency and Ames test mutagenic potency for substances activated by rat liver post-mitochondrial supernatant (S9). The results presented in Figure 2 and models (3) and (4) indicate that 51% to 66% of the variation in mutagenic potency can be explained using SOS genotoxic potency and *Salmonella* strain. In contrast to models (1) and (2), a significant interaction between SOSIP and *Salmonella* strain was detected. Again, the remaining unexplained variation in mutagenic potency can likely be attributed to a wide variety of methodological variations that can effect potency measurements. However, models (3) and (4) have r^2 values that are 10-20% lower than the corresponding values for models that examined direct acting substances. This is likely due to the fact that, in addition to the aforementioned methodological factors, variations in activation mixture preparation and use can have substantial effects on potency measurements (Maron and Ames, 1983; Mersch-Sundermann *et al.*, 1993). Although all studies from which data were collected used an Aroclor 1254-induced rat liver activation system, S9 batch, S9 age and storage conditions, cofactor concentration and final S9 concentrations can also effect potency measurements. Most studies did not conduct experiments to determine the optimum level of S9 required to activate the substance(s) studied. In most cases, the recommended amount of S9 was added to the top agar - 20 μ L per 2.7 mL of top agar i.e. final concentration = 0.75% v/v (Maron and Ames, 1983). However, several studies used more than the recommended amount of S9 (e.g. 50 μ L per plate by Sakai *et al.*, 1985) or failed to indicate the final S9 concentration (e.g. Gurtoo *et al.*, 1978).

Models (5) and (6) relate the maximum mutagenic potency of each substance, regardless of strain, to SOS genotoxic potency. The coefficient of log SOSIP in model (5) (direct acting substances) is not significantly different from the value in models (1) and (2). However, the r^2 value for model (5) is 7% lower than for model (2). Although this model can be used to predict mutagenic potency when strain induced variations are not of interest,

standard errors on individual predictions will be slightly higher. The coefficient of SOSIP in model (6) (S9 activated substances) is significantly greater than two of the coefficients in model (3). In addition, despite the removal of variables that can account for potency variations due to strain type, the r^2 of model (6) is 5% greater than the r^2 of model (4). This may be due to the fact that many of the studies from which the mean mutagenic potency values were calculated did not use an optimal concentration of S9 post-mitochondrial supernatant. Selecting the maximum mutagenic potency values for each compound and using this value as the dependant variable partially removes the variability introduced by sub-optimal S9 concentration.

While some substances (e.g. nitroarenes, *Valerianaceae* roots constituents) have been singled out for their deviation from the models presented, the residual values of complex mixtures from models (1) through (6) were frequently less than the standard error of the estimate (root mean square error of the model). The lack of fit of particular pure substances to the models may provide some insight into the specific mechanistic nuances of mutation formation. In contrast, the 'good fit' of complex mixtures may indicate that short-term assays integrate the total genotoxic effects of the mixture components.

True validation of the models' ability to predict the mutagenic potency of both pure substances and complex mixtures would require new, simultaneous measurements of genotoxic and mutagenic potency. While this was not done in the present study, models (1) and (5) were used to predict the *Ames* mutagenic potency of several genotoxic industrial effluent extracts. The results presented in Table 3 indicate that the predicted values are consistent with values compiled by Houk (1992). The model (5) predictions are all within a factor of 4 of the maximum and mean mutagenic potency values compiled from Houk's data. Model (1) predictions for Group 2 strains (less sensitive) of *Salmonella* (TA1537, TA1535 and TA98) are closest to the minimum mutagenic potency values listed by Houk. While the correspondence between the predicted potency values and the literature potency values is interesting, it should be noted that the comparisons are based on measurements of effluents from different industries that employ a wide range of production and waste treatment techniques.

Models (7) and (8) investigated the quantitative relationships between genotoxic potency and carcinogenic potency for 56 substances that are positive in both assay systems. Few researchers have attempted to investigate the quantitative relationship between genotoxic potency and carcinogenic potency across a diverse array of substances. This is in part due to the lack of a standardized database of carcinogenic potency in the late 1970's when bacterial mutagenicity tests first experienced a large upsurge in use and validation (e.g. McCann *et al.*, 1975; Rinkus and Legator, 1979). Most investigations of the

relationship between carcinogenicity and mutagenicity were qualitative rather than quantitative (e.g. see Odashima, 1976; Poirier, 1976; McCann *et al.*, 1976; Purchase *et al.*, 1976; Rinkus and Legator, 1979; Bartsch *et al.*, 1980; deSerres and Ashby, 1981; Dunkel *et al.*, 1985; Kier *et al.*, 1986; Haseman and Clark, 1990; Kier, 1988; Zeiger, 1987; Tennant *et al.*, 1987; Claxton *et al.*, 1988). Parodi *et al.* (1991) indicated that quantitative correlations can be divided into pre-1985 work and post-1985 work. Studies conducted before 1985 had to generate their own databases of carcinogenic potency values and as a result used small datasets with similar substances (e.g. Meselson and Russel, 1977; Ashby *et al.*, 1982; Zeiger and Sheldon, 1978a; Zeiger and Sheldon, 1987b; Lee and Guttenplan, 1981). Studies conducted after 1985 could make use of the Carcinogenic Potency Database compiled by Lois Gold and colleagues (McCann *et al.*, 1988; Piegorsch and Hoel, 1988; Hatch *et al.* 1992).

Using data on 56 SOS genotoxins that are also listed as significant positives in the Carcinogenic Potency Database we established a significant negative effect of SOSIP on TD₅₀. Thus a smaller dose of a highly genotoxic substance is required to induce tumours in half of the animals in a given experiment. Analysis of covariance indicated that the effect of genotoxicity covaries with experimental animal and route of exposure. Model (7) indicates that almost 30% of the variation in carcinogenic potency can be accounted for by genotoxic potency, animal and route of exposure. The unexplained variation in carcinogenic potency can likely be attributed to a large number of factors including target site, animal sex and animal strain (Gold *et al.*, 1993). In addition, nongenotoxic contributions of cell death and proliferation, induced by chronic and acute toxicity, to neoplasm initiation and promotion can be substantial (McCann *et al.*, 1988; Farber, 1984; Ames, 1989). The intercept values associated with model (7) suggest that rats and primates are more sensitive to neoplasm formation than mice and hamsters. In addition, water, intraperitoneal injection and intravenous injection are more effective routes of administration than exposure through food or air.

Table 4 summarizes the degree of correlation between genotoxic or mutagenic potency and carcinogenic potency observed in this and other studies. Except for the correlation published by Meselson and Russel (1977), the correlation coefficients obtained in this study are similar those determined in other previously published studies (e.g. McCann *et al.*, 1988; Piegorsch and Hoel, 1988; Parodi *et al.*, 1982 and Parodi *et al.*, 1983). Only one study (Piegorsch and Hoel, 1988) actually calculated the slope of the regression function relating mutagenic and carcinogenic potencies. Their slope value (0.223) is not significantly different from the slope values in models (7) and (8).

Model (8) was used to predict the carcinogenic potency of the most genotoxic industrial effluent extract encountered in a previous study of 150 extracts from 50 industrial effluents (White *et al.*, 1995 - see Table 3). The predicted maximum carcinogenic potency (as TD₅₀) is 122 ± 54 mg per kg body weight per day. This value places the most potent industrial effluent extract examined by White *et al.* (1995) among the fairly weak carcinogens (e.g. safrole, captan and styrene oxide). The predicted maximum carcinogenic potency values of the previously examined industrial effluent extracts generally exceed 10^3 mg per kg body weight per day.

The goal of this study was to produce quantitative, empirical relationships that permit SOS Chromotest results to be interpreted in a much broader empirical framework. This framework was established via the construction of models that permit the prediction of *Ames* mutagenic potency and animal carcinogenic potency from SOS genotoxic potency values. The models demonstrate strong relationships between both direct acting and S9 mediated mutagenicity and SOS genotoxicity. While the relationship between genotoxicity and carcinogenicity is distinctly weaker, it is consistent and significant. The models presented can be used to predict the potential mutagenicity and carcinogenicity of pure substances and complex environmental extracts tested using the SOS chromotest. Further research could investigate the mechanistic causes underlying the empirical relationships described. Several researchers have identified a variety of physical-chemical attributes that determine genotoxicity, mutagenicity and carcinogenicity (see Debnath *et al.*, 1992a; Debnath *et al.*, 1992b; Debnath and Hansch, 1992; Zhang *et al.*, 1993, Hatch *et al.*, 1992; Mercsh-Sundermann *et al.*, 1992b). Whether or not similar physical-chemical attributes are responsible for the genotoxicity, mutagenicity and carcinogenicity of the pure substances and complex mixtures listed in appendix 4 remains to be determined. The data sets used here, in combination with a database that describes the physical-chemical attributes of the pure substances examined, could be used to address this question.

ACKNOWLEDGEMENTS

We gratefully acknowledge the support and assistance of several people. Philippe Quillardet at the Pasteur Institute, Paris and Audrey McDaniels at the USEPA, Cincinnati for supplying us with unpublished SOS Chromotest data. Alice Hontella at the University of Québec at Montréal for translating some Russian manuscripts. Brian Walker for finding a large fraction of the data and digitizing most of the figures. Judy Adam for ensuring that the reference list is complete and correct. This work was supported by a Natural Sciences and Engineering Research Council of Canada operating grant to JBR and a Vineberg Family Fellowship (McGill University) to PAW.

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Note: Many of the citations below refer to the data sources listed in Appendix 4.

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CHAPTER 3

COMPARING THE PRESENCE, POTENCY AND POTENTIAL HAZARD OF GENOTOXINS EXTRACTED FROM A BROAD RANGE OF INDUSTRIAL EFFLUENTS

INTRODUCTORY REMARKS

Chapter 1 described a rapid and effective version of the SOS Chromotest for the analysis of complex environmental samples. Chapter 2 investigated empirical relationships between SOS genotoxicity and two more familiar endpoints. The next logical step in the research project is the use of the SOS Chromotest to investigate sources of genotoxic organic contaminants. This chapter presents the results of SOS Chromotest analyses of a wide range of industrial effluents that are discharged into the St. Lawrence and Saguenay rivers.

The material presented is *in press* in volume 26 of *Environmental and Molecular Mutagenesis*. Both Prof. Joseph Rasmussen and Dr. Christian Blaise played a role in the development of the ideas presented. As such, they are second and third co-authors respectively.

Appendix 5 contains the actual names and locations of the industries listed in Table 1. Appendix 6 contains the data used to generate the models, figure and tables in chapters 3 and 4.

ABSTRACT

We examined the genotoxicity of dichloromethane extracts from 50 final effluent samples collected from 42 industries- including pulp & paper, chemical manufacturing, metal refining, metal surface treatment and municipal wastewater treatment. Effluents were initially fractionated into dissolved substances and substances adsorbed to suspended particulate matter. Acid/base partitioning was used to further fractionate aqueous extracts. Genotoxicity was measured using the SOS Chromotest. Genotoxicity of extracts was found to be related to sample type, industry type, metabolic activation status and extract fluorescence (380nm excitation, 430nm emission). S9 metabolic activation reduced genotoxic potency in over 90% of the extracts examined. Expression of potency values per equivalent unit of original sample revealed that effluent particulate matter is, on average, almost 4 orders of magnitude more potent than the aqueous filtrates. Suspended particulate matter from organic and inorganic chemical production, petroleum and metal refining as well as metal surface treatment facilities provided extracts that are significantly more genotoxic than those from sewage treatment and pulp & paper facilities. Aqueous filtrates from inorganic and organic chemical production, metal refining and surface treatment facilities are significantly more genotoxic than those emitted by aluminum and petroleum refineries. Overall the results suggest that pulp & paper mills emit mostly soluble genotoxins, while petroleum and aluminum refineries emit predominantly particle-associated genotoxins. Although some extracts elicited a strong SOS response, the potencies of the extractable residues are low when compared to highly potent pure substances such as benzo(a)pyrene. On average, a mg of dichloromethane extractable residue has an SOS genotoxicity equivalent to 0.1-1.0 μg of benzo(a)pyrene. Predicted Ames mutagenic potency values correspond reasonably well with industrial waste mutagenic potency values published by other researchers. Genotoxic loading values were calculated to quantify the total daily genotoxic emission and potential hazard of each industry. Highest loadings are from sewage treatment, pulp & paper and metal refining facilities. Highest loading values are the SOS genotoxic equivalent of over 30 kg of benzo(a)pyrene per day. The ultimate hazard of the genotoxic emissions is not known. Actual hazard assessment is complicated by a poor understanding of the post-emission behavior of genotoxins. Exposure of downstream biota is likely substantial.

INTRODUCTION

Modern industries generate large quantities of hazardous waste. In Canada alone approximately 6.5 million metric tons of hazardous waste are generated each year (1992 estimate- Environment Canada, 1988a). These pollutants frequently enter the aquatic ecosystems of rivers, lakes and harbors via direct, aqueous emissions. The consequences of these emissions can be serious. Many researchers have detected an abnormally high frequency of fish neoplasia in industrialized regions (Black and Baumann, 1991; Vogelbein et al., 1990; Mix, 1986; Krahn et al., 1986; Malins et al., 1984; Kinne et al., 1981; Pierce et al., 1978; Sonstegard, 1977; Brown et al., 1973). In an effort to protect the aquatic environment, government agencies in several countries have adopted legislation aimed at reducing or even eliminating toxic, industrial discharges. The first step towards achieving this goal is the assessment of effluent toxicity and its potential, post-emission hazard.

Effluent toxicity or potential hazard is frequently assessed via chemical analyses and/or laboratory bioassays. The use of bioassays has focussed to a large extent on acute toxicity. However, acute toxicity only represents the first level of impact on the aquatic ecosystem (Van Coillie et al., 1989; deRaaf et al., 1985). It is now well established that many industrial discharges contain substances that may have no acute effect, but are capable of reducing the long term survival of an organism via damage to the genome of somatic and germ cells (see review by Houk, 1992). Such genetic damage has been linked to heritable genetic disorders and cancer (Metcalf et al., 1990; Couch and Harshbarger, 1985; McBee et al., 1987). Detailed chemical analysis is limited in its ability to predict the toxicity of complex mixtures (Brouwer et al., 1990; Samoiloff, 1983; Houk, 1992). In addition, the toxicity of unaltered effluents or organic extracts can rarely be accounted for by the concentration of identified toxicants (Ellis et al., 1982). An alternative approach involves the fractionation of complex extracts into chemically distinct mixtures each of which can subsequently be examined for toxicity (Lewtas, 1988; Alfheim et al., 1984; Houk, 1992). This type of bioassay-directed fractionation has been used to determine the chemical nature of mutagens and genotoxins in a wide variety of industrial and municipal wastewaters (Somani et al., 1980; Meier et al., 1987; Epler et al., 1978; Donnelly et al., 1983).

The bioassay that is frequently used to assess the genotoxicity of industrial waste samples is the *Salmonella*/mammalian microsome assay or Ames test (see review by Houk, 1992). Since the advent of this test, several other short-term bacterial genotoxicity assays have been developed. Several of these assays (e.g. Microscreen phage induction

Assay, SOS/*umu* test, SOS Chromotest) exploit *Escherichia coli*'s ability to respond to DNA damage events via the SOS response pathway (Walker, 1984). The SOS Chromotest was first described by Quillardet et al. (1982). Since its introduction, three validation studies (Ohta et al., 1984; Quillardet et al., 1985; Von der Hude et al., 1988) and one review (Quillardet and Hofnung, 1993) have demonstrated good correspondence between the SOS Chromotest and the more traditional Ames/*Salmonella* test. In addition, several studies indicate that the test (and/or response pathway) is useful for monitoring the genotoxicity of complex environmental samples (White et al., 1995a; Qian et al., 1994; Ong et al., 1986; Courtois et al., 1988; Schleibinger et al., 1989; Harwood et al., 1989; Houk and deMarini, 1988; Whong et al., 1986).

While bioassay-directed fractionation has some distinct advantages over routine chemical characterization of priority substances (Schuetzle and Lewtas, 1986), neither chemical characterization nor (geno)toxicity assessment can assess the risk of inhabiting downstream regions that receive (geno)toxic industrial waste. Bioassays permit the assessment of waste toxicity under controlled laboratory conditions. However, risk is the likelihood that the potential hazard of the toxic mixture will actually be realized (Paasivirta, 1990). Measurement of post-emission exposure and relative risk for *in situ* (geno)toxic effect is difficult. However, since the relative risk of effect increases with increasing exposure (Paasivirta, 1990; Valcovic, 1991), potential hazard can be estimated by combining (geno)toxicity results with daily, volumetric emission rates.

This study investigated the genotoxicity of organic extracts from 50 industrial effluent samples. The industries investigated represent a wide range of industrial processes including metal refining (Al, Cu, Ti, Cr, Zn, Ag, Au, Pt and Pd), paper manufacturing, metal surface treatment, inorganic chemical manufacture, organic chemical manufacture, petroleum refining and municipal wastewater treatment. Since organic toxicants frequently have a high affinity for suspended particulate matter (Karickhoff et al., 1979; Allan, 1986), each effluent was separated into suspended particulates and aqueous filtrate prior to extraction. Organic extracts of both the particle-associated and aqueous phases were prepared by direct partitioning into dichloromethane. Genotoxicity was measured using a semi-automated version of the SOS Chromotest. Acid/base partitioning of aqueous filtrates was employed to yield further information about the chemical nature of the putative genotoxins. Daily genotoxic loading rates were calculated from SOS Chromotest results and daily discharge measurements.

MATERIALS AND METHODS

Industry Selection and Effluent Collection-

Industries selected for analysis are recognized by Environment Canada and the environment ministry of the province of Québec as priorities for concern and control. In addition, many of the selected industries belong to categories that have been recognized by the World Health Organization (IARC, 1989a; IARC, 1989b; IARC, 1984; IARC, 1981) as sources of genotoxic and carcinogenic compounds. Forty of the industries are located in the province of Québec and discharge their waste into the St. Lawrence river or Saguenay river. The remaining two industries are located in the province of British Columbia and discharge their waste into the Fraser river.

Thirty-six liters of effluent were collected from each industry. Samples were collected using an automatic sampler (Manning Environmental Corp., Santa Cruz, CA) that collects 375 mL of wastewater every 15 minutes for 24 hours. Therefore, each sample was a 24 hour integrated sample. Samples were collected, transported and stored according to the guidelines outlined by Vezeau (1982). Sub-samples for genotoxicity analyses were stored in pre-cleaned containers at 4°C. Final effluent samples were collected as close as possible to the point of discharge into the receiving water. Where no single final effluent existed, composite samples were prepared. Composite samples contained all final effluent streams mixed in proportion to their discharge. During the sampling period, final effluent flow was measured using a Parshall flume (Plasti-Fab Inc., Tualatin, OR). In total, 50 final effluent samples were collected from 42 industries. Table 1 provides a list of the industries investigated, the principal product produced, the mean daily discharge and the concentration of suspended particulate matter.

Effluent Processing and Extract Preparation-

Suspended particulates from 4-10L of effluent were collected on 142 mm glass fibre filters (1µm retention Type AE filters- Gelman Science, Ann Arbor, MI) via positive pressure filtration under ultrapure nitrogen gas. Filters were stored at -20°C until extraction. Filtrates were divided into two sub-samples. One sub-sample was adjusted to pH 2 with 6N HCl and extracted with 400-500mL of pesticide grade dichloromethane (Anachemia Science, Montréal). The second sub-sample was adjusted to pH 12 with 5N NaOH prior to extraction with dichloromethane. Although other solvents have been used for effluent extraction, dichloromethane has been called the solvent of choice for the analysis of trace, non-polar organics in both solid and liquid samples (Hunchak and Suffet, 1987). In addition, dichloromethane is often used to extract genotoxic organics

Table 1. Industries investigated. Company names have been omitted to ensure anonymity[†]. Principal product, mean discharge (m^3 per min - based on 2-5 day monitoring period) and concentration of suspended particulate matter (mg dry matter per L) for each industry. Samples were final effluents except where indicated. FI indicates 24 hr. integrated final effluent sample; FIC indicates 24 hr integrated, composite effluent sample. Composite samples were prepared where no single final effluent existed. Composites contained individual final effluent streams mixed in proportion to their discharge. NM indicates not measured. With the exception of Inorganic #3, Metal #3 and Surface #3 for which two separate waste streams were sampled, the second sample represents the same final effluent sampled at a later date.

Industry Name	Principle Product	Discharge ($\text{m}^3 \text{ min}^{-1}$)	TSP [∞] (dry mg L ⁻¹)	Sample Type
<u>Aluminum Refineries--</u>				
Aluminum #1	Aluminum ingots	0.80	10.7	FI
Aluminum #2- Sample 1	Aluminum ingots	3.92	15.0	FI
Aluminum #2- Sample 2	Aluminum ingots	3.85	0.6	FI
Aluminum #3	Aluminum ingots	9.18	1.0	FIC
Aluminum #4	Aluminum ingots	0.17	5.0	FIC
Aluminum #5	Aluminum foil	0.52	21.3	FIC
Aluminum #6- Sample 1	Aluminum ingots	7.95	31.0	FIC
Aluminum #6- Sample 2	Aluminum ingots	3.85	3.6	FIC
<u>Inorganic Chemical Production Facilities--</u>				
Inorganic #1	Elemental phosphorus	4.68	14.4	FI
Inorganic #2	Chlorine & Alkali	4.58	12.0	FIC
Inorganic #3- Sample 1	Titanium dioxide	NM	69.0	FI*
Inorganic #3- Sample 2	Titanium dioxide	NM	73.5	FI
Inorganic #4	Chlorine & Alkali	9.90	4.0	FI
Inorganic #5- Sample 1	Titanium dioxide	5.80	259.3	FI
Inorganic #5- Sample 2	Titanium dioxide	5.83	199.0	FI
<u>Non-Aluminum Metal Refineries--</u>				
Metal #1	Stainless steel	54.17	58.0	FIC

Table 1 continued.....

Metal #2	Refined Cu, Ag, Au, Pt & Pd	14.24	2.6	FIC
Metal #3- Sample 1	Zn and Cd Ingots	111.15	4.6	FI
Metal #3- Sample 2	Zn and Cd Ingots	2.74	15.7	FI [†]
Metal #4	Nb dioxide	3.82	7.8	FIC
Metal #5	Steel shot, cast iron, Ti slag	125.00	1279.0	FIC
Metal #6	Stainless steel	31.46	23.0	FI

Organic Chemical Production Facilities--

Organic #1	Ethanol, methanol, butanols	0.63	15.0	FI
Organic #2	Polyvinyl acetate	1.72	12.0	FI
Organic #3	Preserved wood products	0.02	2.0	FI
Organic #4	Organic polymers and herbicides	1.13	246.0	FIC
Organic #5	Industrial adhesives, plastic resins	0.76	24.2	FI
Organic #6- Sample 1	High density polyethylene, specialty chemicals	3.30	29.0	FI
Organic #6- Sample 2	High density polyethylene, specialty chemicals	3.30	29.2	FI
Organic #7	Explosives	14.08	2.6	FIC

Pulp and Paper Production--

Paper #1	Specialty paper products	92.32	31.0	FI
Paper #2	Newsprint	26.74	92.3	FI
Paper #3	Kraft paper products	86.12	82.2	FI
Paper #4	Hygienic paper products	2.80	9.3	FI
Paper #5	Specialty paper products	7.50	162.0	FI
Paper #6	Kraft paper products	53.47	1484.0	FIC

Petroleum Refining Facilities--

Petroleum #1- Sample 1	Refined petroleum prod.	12.71	33.0	FI
Petroleum #1- Sample 2	Refined petroleum prod.	9.03	29.0	FI

Table 1 continued.....

Petroleum #2	Refined petroleum prod.	1.46	24.0	FI
Petroleum #3	Refined petroleum prod.	7.24	16.8	FI
Petroleum #4- Sample 1	Refined petroleum prod.	6.57	32.1	FI
Petroleum #4- Sample 2	Refined petroleum prod.	6.57	32.3	FI

Municipal Wastewater Treatment Plants--

Sewage #1	Primary treatment- municipal waste	288.80	61.5	FI
Sewage #2	Primary treatment- municipal waste	1122.00	19.0	FI

Surface Treatment Facilities--

Surface #1	Ni, Cd and Ni plated products	0.31	9.2	FI
Surface #2	Galvanized steel	0.05	295.0	FI
Surface #3- Sample 1	High performance engine parts	0.60	11.0	FI ^Δ
Surface #3- Sample 2	High performance engine parts	1.45	64.0	FI
Surface #4	Reconditioning of used aircraft engines	1.74	14.8	FI
Surface #5	Reconditioning of used aircraft engines	0.02	9.0	FI

[‡]Full names can be found in Appendix 5

[∞]Total suspended particulate matter

* sulphate plant effluent

† acid neutralization effluent

^Δ intake water

from a variety of waste samples (Montreuil et al., 1992; USEPA, 1985; Tabor and Loper, 1987). All extractions were performed in a continuous, high-volume, liquid-liquid extractor. Design and operation of the extractor is outlined in Goulden and Anthony (1985). Extensive validation studies have determined that the instrument provides excellent recovery of a variety of semi-volatile, trace organics including pesticides (60-100% recovery- Foster et al., 1993; Stevens and Neilson, 1989), PCBs (65-100%- Stevens and Neilson, 1989; Kuntz et al., 1992) and polycyclic aromatic hydrocarbons (64-100%- Kuntz et al., 1992). Particulate matter concentrations (dry mg per L) were

determined by gravimetric analysis (APHA, 1989). Particulate matter present in 100-500 mL effluent aliquots was collected on preweighed glass fibre filters. The filters were dried overnight at 105°C and reweighed.

Suspended particulates were extracted using a combined blender/sonication method. The method is a variation on those developed by Marble and Delfino (1988), Maggard et al. (1987) and Williams (1989). Maggard et al. (1987) determined that, for mutagenicity studies, the performance of blender methods is similar or better to that obtained using Soxhlet extraction. Briefly, filters were blended with 400-500mL of dichloromethane in stainless steel cups using a high-speed (3500 rpm) industrial blender (Eberbach Corp., Ann Arbor, MI). Each sample was blended for 3 x 4 minutes and subsequently sonicated (ultrasonic cell disrupter- Branson Sonic Power Co., Danbury, CT) on ice for 2 x 3 minutes. Marvin et al. (1994) determined that the addition of a second sonication step, can increase the yield of polycyclic aromatic substances by up to 18%. Blending and sonication periods were alternated with 3-4 minute cooling periods. The blender/sonication method was validated by spiking an uncontaminated suspended sediment sample with a mixture of 15 polycyclic aromatic hydrocarbons (Supelco PAH Mix 4-8905, Supelco Canada Inc., Oakville, Ontario). Mean recoveries of 3-, 4- and 5-ring PAHs were 65%, 80% and 89% respectively.

All extracts were dried using anhydrous sodium sulfate (Mallinckrodt Specialty Chemicals, Mississauga, Ontario) and reduced to approximately 5 mL by rotary evaporation at 30°C. Suspended particulate extracts were filtered through a coarse, sintered glass funnel to remove filter fragments and particulate matter. All extracts were taken to dryness under vacuum at low temperature (Speedvac AS290 Concentrator, Savant Ltd., Farmingdale, NY). Prior to resuspension, the mass of the extractable residue was determined gravimetrically. Final extracts were divided in two. One half was resuspended in 100µL high purity dimethyl sulfoxide (DMSO) (Sigma Chemical Co., St. Louis, MO) for genotoxicity analyses. The other half was resuspended in 3 mL of dichloromethane for measurement of extract fluorescence via fluorescence spectrophotometry. Ultrapure (Super Q™- Millipore Corp., Bedford, MA) laboratory grade water was used as a matrix blank.

All labware was precleaned with pesticide grade acetone and hexane. Glassware was baked at 350°C to remove solvent traces and covered with pre-cleaned aluminum foil. Glass fibre filters and sodium sulfate were combusted at 450°C before use.

SOS Chromotest-

Genotoxicity was measured using the SOS Chromotest (Quillardet et al., 1982;

Quillardet and Hofnung, 1985). The test involves incubation of *Escherichia coli* PQ37 (*sulA::Mud(Ap lac)* cts *lacΔU169 mal⁺, uvrA, galE galY, Pho^C, rfa*) with a pure compound or complex mixture. The *sulA::Mud(Ap lac)* fusion places the production of functional β -galactosidase under the express control of the SOS response pathway which is induced by DNA damaging agents (Walker, 1984). Post-exposure β -galactosidase activity reflects the genotoxicity of a given sample. Alkaline phosphatase activity provides an indirect measure of cell viability (Quillardet et al., 1982). Each extract was tested both in the presence and in the absence of a post mitochondrial supernatant (S9 fraction) obtained from Arochlor 1254 induced rats (Molecular Toxicology Products Inc., Annapolis, MD). S9 mixture was prepared following the method of Quillardet and Hofnung (1985). Final S9 concentration was 1% v/v. 4-Nitroquinoline-1-oxide and 2-aminoanthracene were used as positive controls.

The protocol employed is a variation on that described by Quillardet and Hofnung (1985). To permit rapid analysis of a large number of samples, the assay was carried out in microtiter plates and automated using the Biomek™ 1000 Automated Laboratory Workstation (Beckman Instruments, Palo Alto, CA). The details of the method and its validation using extracts of a series of standard reference materials will be discussed elsewhere. Briefly, a 16 hour overnight culture (37°C) of *E. coli* PQ37 was diluted in fresh medium (Luria-Bertani (LB) broth + 20 μ g per mL ampicillin) to an optical density (600nm) of 0.05-0.075. The sample (or solvent blank), dissolved in 10 μ L DMSO was then added to 190 μ L of diluted culture (plus S9 if desired) present in the first well of each dilution series. An automated multichannel pipette was then used to mix the well contents and perform a 2-fold serial dilution with an adjacent well that already contains 95 μ L of diluted culture and 5 μ L of DMSO. The two-fold serial dilution was continued in four additional wells for a total of six, 2-fold serial dilutions. This dilution system permitted full use of the automated multichannel pipette, while not permitting any change in the concentration of carrier solvent or cells. Twelve control wells received PQ37 culture and a solvent blank. Each extract was tested in duplicate at the highest possible concentration range. The final background concentration of DMSO was 5% v/v.

Microplates were incubated for 3 hours at 37°C and subsequently centrifuged at 2000g for 20 minutes. The supernatant was removed and the bacterial pellet resuspended in 100 μ L of 200mM (Tris[hydroxymethyl])-aminomethane pH 7.5 and 100 μ L of cell lysis/enzyme assay buffer (40% v/v methanol, 0.5% v/v toluene, 10% v/v N,N-dimethyl formamide, 4mg/mL 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-Gal), 1mg/mL *p*-nitrophenyl phosphate). Centrifugation and supernatant removal permitted the removal of colored substances that can contribute to the sample's optical density and

complicate enzyme activity measurements. Plates were mixed for 60 seconds (Titertek microplate vortex, Flow Laboratories, Lugano, Switzerland) and incubated (37°C) for 60 minutes after which *initial* optical density readings (Titertek Multiskan MCC/340 Microplate Spectrophotometer) were taken (620 nm and 405 nm). Final optical density readings were taken 120 minutes after cell resuspension and lysis. Optical density values recorded at 620nm measure the activity of β -galactosidase, those recorded at 405nm measure the activity of alkaline phosphatase. Calculation of net optical density values (120 min value - 60 min value) allowed the contribution of any remaining colored substances to be accounted for prior to analysis of the results.

Data Analyses-

Induction of the SOS response pathway at a sample concentration C, denoted R(C), is the ratio of net (120 min - 60 min) β -galactosidase activity to net alkaline phosphatase activity. To account for the background induction of the *sulA* gene, a normalized SOS response induction factor was calculated as $IF = R(C)/R(0)$, where R(0) is the ratio of β -galactosidase to alkaline phosphatase activity averaged over the 12 controls which received blank solvent. A normalized induction factor that exceeded the upper 95% confidence limit of the control was regarded as a significant positive. Since normalized induction factor values are ratios of two means, confidence limits were calculated according to Welsch et al. (1988). Welsch et al. (1988) describe methods for calculating the variance associated with various functions of mean values (reciprocals, sums, products, ratios, etc). In the case of a ratio, the variance of the ratio is calculated from the mean and variance of the numerator and the denominator as well as their respective reciprocals. Concentration response curves of normalized induction factor against sample concentration (mg sample equivalent per well) were inspected and the results qualitatively categorized as follows-

- 1) Negative- induction factor never exceeded the upper confidence limit of the control;
- 2) Marginal- induction factor exceeded the upper confidence limit of the control at one or two concentrations only;
- 3) Positive- induction factor exceeded the upper confidence limit of the control at a minimum of three doses;
- 4) Erratic- induction factor exceeded the control at three or more concentrations, but the concentration-response relationship was highly erratic and could not be interpreted;
- 5) Miscellaneous- special cases where the concentration response data could not be plotted (e.g. accurate serial dilution impossible due to extract viscosity and

insolubility).

Every effort was made to remove erratic responses from the data set. All samples that produced erratic responses, especially where growth inhibition was substantial, were reanalyzed at one-tenth and/or one-hundredth the original concentration range.

Three genotoxicity parameters were calculated for each significant, positive response. For simplicity, the acronyms used here are similar to those described by Langevin et al. (1992). SRIP- the SOS response inducing potency, is the initial slope of the concentration response curve. The SRIP is expressed in SOS Induction factor units per equivalent mg of original sample (i.e. filtrate or particulate matter). Potency was also expressed as RES-SRIP- the SOS response inducing potency in SOS Induction factor units per mg of dichloromethane-extractable residue. GTOX- a measure of the degree of contamination of a given extract with SOS genotoxins was calculated as the inverse of the MDGC value of Langevin et al. (1992). MDGC is the minimum detectable genotoxic concentration (in mg sample equivalent). The concentration of extract, inferred from the fitted concentration-response curve, required to produce an induction factor that equals the upper 95% confidence limit of the control. GTOX values are expressed in mg sample equivalent⁻¹. The GTOX unit is similar in concept to *toxic units*, a parameter originally proposed by Sprague and Ramsay (1965) and used to assess the contamination of industrial effluents with acute and chronic toxicants (Costan et al., 1993).

When concentration response curves were distinctly nonlinear, exhibiting zero-order kinetics at high sample concentration, the concentration response curves were analyzed by iterative, non-linear regression employing a least squares loss function (SAS Institute, 1987). In these cases, SRIP and MDGC were calculated according to Langevin et al. (1992). When the range of concentrations tested did not result in a full hyperbola, the SRIP and MDGC were determined by ordinary, least squares linear regression.

All data analyses were performed using SAS version 6.08. Analysis of variance (ANOVA), analysis of covariance (ANCOVA) and least squares regression analysis were used to investigate the effect of sample type, industry type, metabolic activation status and extract fluorescence on sample genotoxicity. Where necessary, the data were log transformed to equalize the variance over the range of observations and meet the normality assumption of least squares regression and analysis of variance (Ricker, 1973). The residual error associated with all regression, ANOVA and ANCOVA models was assumed to be normally distributed with a mean of zero and variance σ^2 . Normality of residual error was assessed using the Komorogov-Smirnov test (Zar, 1984) and visual inspection of a normal probability plot (SAS Institute, 1987).

Fluorescence Spectrophotometry Analyses-

Attempts to relate the genotoxicity of complex mixtures to the concentration of identified, priority substances has met with varying degrees of success. In many cases, detailed chemical analysis failed to detect the putative genotoxins and little or no relationship was found between extract genotoxicity and the concentration(s) of identified substances (Houk, 1992; Jungclaus et al., 1978; Wright et al., 1989; Langevin et al., 1992; Sato et al., 1983; Grifoll et al., 1990). In this study, fluorescence spectrophotometry was employed to provide a non-specific chemical characterization of each extract. Benzo(*a*)pyrene (BaP) was used as a fluorescence standard and all results are expressed in ppb (μg per L liquid or ng per g solids) of BaP required to produce fluorescence equivalent to a given sample. Excitation/emission wavelengths used are the BaP wavelengths of Krahm et al. (1984)- 380nm excitation and 430nm emission. At these wavelengths, a large portion of the total fluorescence will be determined by substances with molar absorptivities that are similar to benzo(*a*)pyrene. These substances include 5- and 6-ring polycyclic aromatic substances such as dibenzo(*ai*)pyrene and benzo(*ghi*)perylene (Friedel and Orchin, 1951). The method used is similar to that employed by Zitko (1975) to assess the contamination of aquatic biota with petroleum hydrocarbons. Zitko expressed fluorescence of aquatic fauna extracts as *pyrene equivalents*. All samples were analyzed using a Hitachi F3010 Fluorescence Spectrophotometer (Hitachi Ltd., Tokyo, Japan) calibrated with a 50 ppb solution of BaP (Sigma Chemicals, St. Louis, MO) in high purity dichloromethane.

The following abbreviations are used in the text: SRIP = SOS response inducing potency (induction factor units per equivalent mg of original sample), GTOX = genotoxic contamination ($\text{mg sample equivalent}^{-1}$), RES-SRIP = SOS response inducing potency expressed in induction factor units per mg of dichloromethane extractable residue, FLUOR = extract fluorescence, expressed as ppb BaP required to produce equivalent fluorescence, TSP = total suspended particulate matter (dry mg per L), BaP = benzo(*a*)pyrene, IF = SOS induction factor.

RESULTS

Three extracts were prepared from each of the 50 effluent samples- a suspended particulate extract, an alkaline aqueous extract and an acidic aqueous extract- providing a total of 150 extracts. Each extract was tested both with and without S9 metabolic activation yielding a total of 300 concentration-response plots. Figure 1 includes concentration-response plots for 8 effluent extracts that elicited a strong positive response. Two of the concentration-response relationships (Petroleum refinery #2 and Petroleum refinery #1-Sample 1) appear completely linear and did not reach zero-order kinetics. The remaining six plots range from mildly to distinctly non-linear in appearance. Overall, 64% of the positive responses were distinctly non-linear in appearance and were analyzed by the method of Langevin et al. (1992). The remaining 36% of positive responses never approached zero order kinetics and were analyzed by linear regression.

Not all samples elicited a positive response. Table 2 contains a qualitative summary of the results obtained. Approximately 48% of the extracts tested elicited a significant, positive response. Addition of S9 decreased the fraction of aqueous samples that elicited a positive response by more than 25%. For all three sample types, the addition of S9 mixture reduced the proportion of responses that were erratic. In addition, suspended solid extracts elicited a positive response more frequently than either of the aqueous extracts.

The results obtained reveal wide variation in both SRIP and GTOX. SRIP and GTOX values for aqueous extracts, both acidic and alkaline, are far lower than the corresponding suspended particulate values. Most extracts elicited lower SRIP and GTOX values when tested in the presence of S9 metabolic activation. Figure 2 illustrates the effect of S9 presence and sample type on mean SRIP. The figure illustrates that the mean potency of suspended particulate extracts is almost four orders of magnitude above that of the aqueous extracts. Responses obtained in the presence of S9 metabolic activation are consistently about an order of magnitude below those obtained without metabolic activation. A similar pattern was identified for GTOX, the parameter that reflects the degree of genotoxic contamination.

Some industry types consistently provided highly genotoxic effluent samples. Figure 3 illustrates the trend in extract potency across industry type. With respect to the aqueous extracts, pulp & paper mills, aluminum refineries, non-aluminum metal refineries and surface treatment industries provided the most potent direct acting samples. Organic chemical production facilities, aluminum refineries and petroleum refining facilities provided the most potent direct acting, particle-associated samples. Again, a similar

Fig. 1. SOS Chromotest concentration-response relationships for 8 of the 150 extracts examined (3 extracts for each of the 50 samples obtained from 42 industries). Concentration values are expressed as mg of the original starting material (liquid filtrate or suspended particulate matter) per microplate well (0.2 mL). Two of the examples are linear and do not reach a plateau at the tested concentrations. The remaining six examples approach a plateau at which point zero order kinetics will be manifested. SOS induction factor values and confidence intervals on the control were calculated as described in the text. Error bars represent one standard error of the mean. Where error bars are not visible, they were smaller than the plotting symbol.

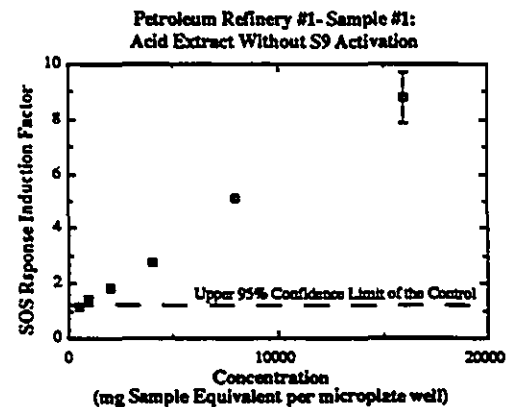
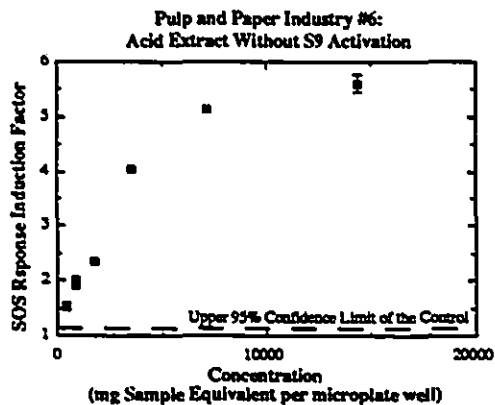
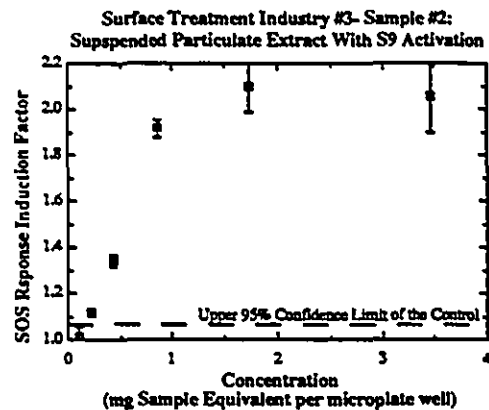
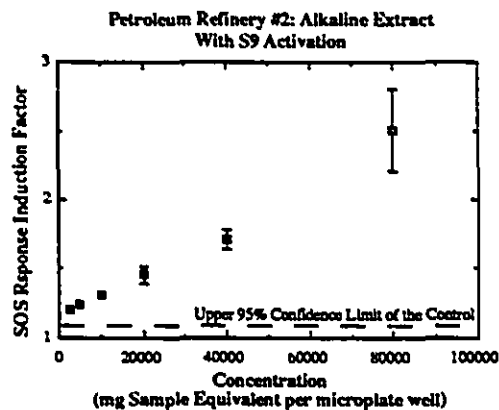
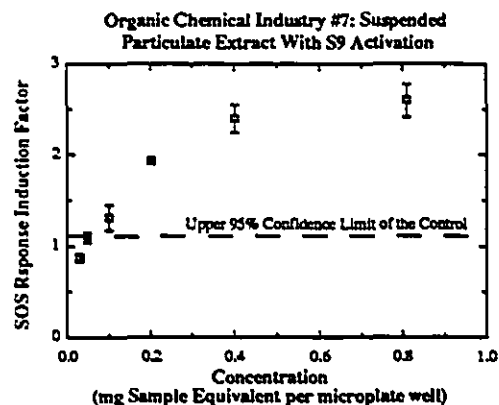
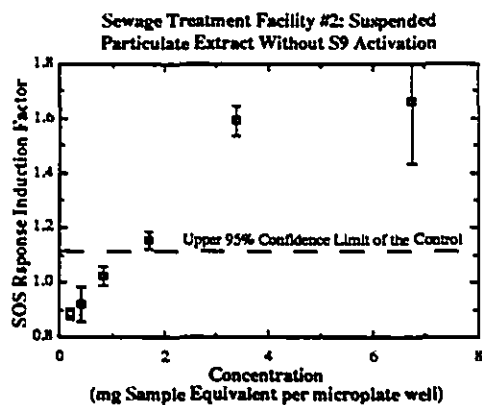
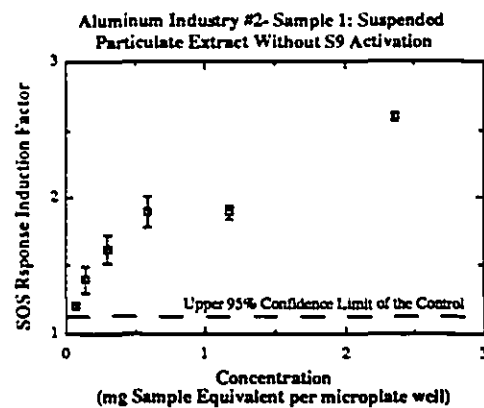
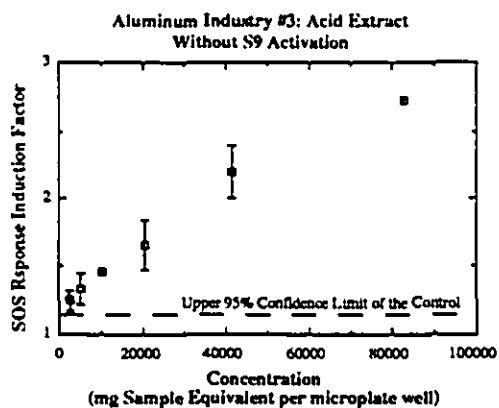


Table 2. Qualitative summary of SOS Chromotest results. Category assignments are as follows: positive- responses which exceeded the upper 95% confidence limit of the control at a minimum of three concentrations. Erratic- those samples which may have exceeded the control, but elicited a highly erratic concentration-response relationship. Negative or marginal- those samples which were unable to invoke a response that exceeded the control at three concentrations. The miscellaneous category includes problematic extracts. For example, the extract from Organic #4 was highly viscous and precipitated immediately on contact with the aqueous bioassay medium. An accurate serial dilution was not possible.

Sample Type	Results Without Metabolic Activation	Results With Metabolic Activation
Extracts of Suspended Solids (n=50)	70% Positive 14% Erratic 10% Negative or marginal 6% Miscellaneous	76% Positive 2% Erratic 18% Negative or marginal 4% Miscellaneous
Acidic Extract of Filtrate (n=50)	62% Positive 26% Erratic 10% Negative or marginal 2% Miscellaneous	22% Positive 6% Erratic 70% Negative or marginal 2% Miscellaneous
Alkaline Extract of Filtrate (n=49)	43% Positive 35% Erratic 20% Negative or marginal 2% Miscellaneous	16% Positive 10% Erratic 71% Negative or marginal 3% Miscellaneous

pattern was identified for the GTOX parameter.

Although S9 activation often decreased both SRIP and GTOX, the magnitude of the effect varied with sample and industry type. In the absence of S9 activation, suspended particulate extracts from organic chemical production facilities provided the most potent response. S9 activation dramatically reduced the mean organic chemical SRIP from 17 IF Units per mg sample to 0.3 IF Units per mg sample. The effect on metal (Al and others)

Figure 2. The effect of S9 metabolic activation and sample type on the genotoxicity of industrial effluent extracts. The effect of S9 metabolic on the mean SRIP of acidic extracts, alkaline extracts and suspended particulate extracts is shown. In each case, analysis of variance revealed a significant effect of S9 presence. A- acidic, aqueous extracts; B- alkaline, aqueous extracts; C- suspended particulate extracts. Error bars are one standard error of the mean.

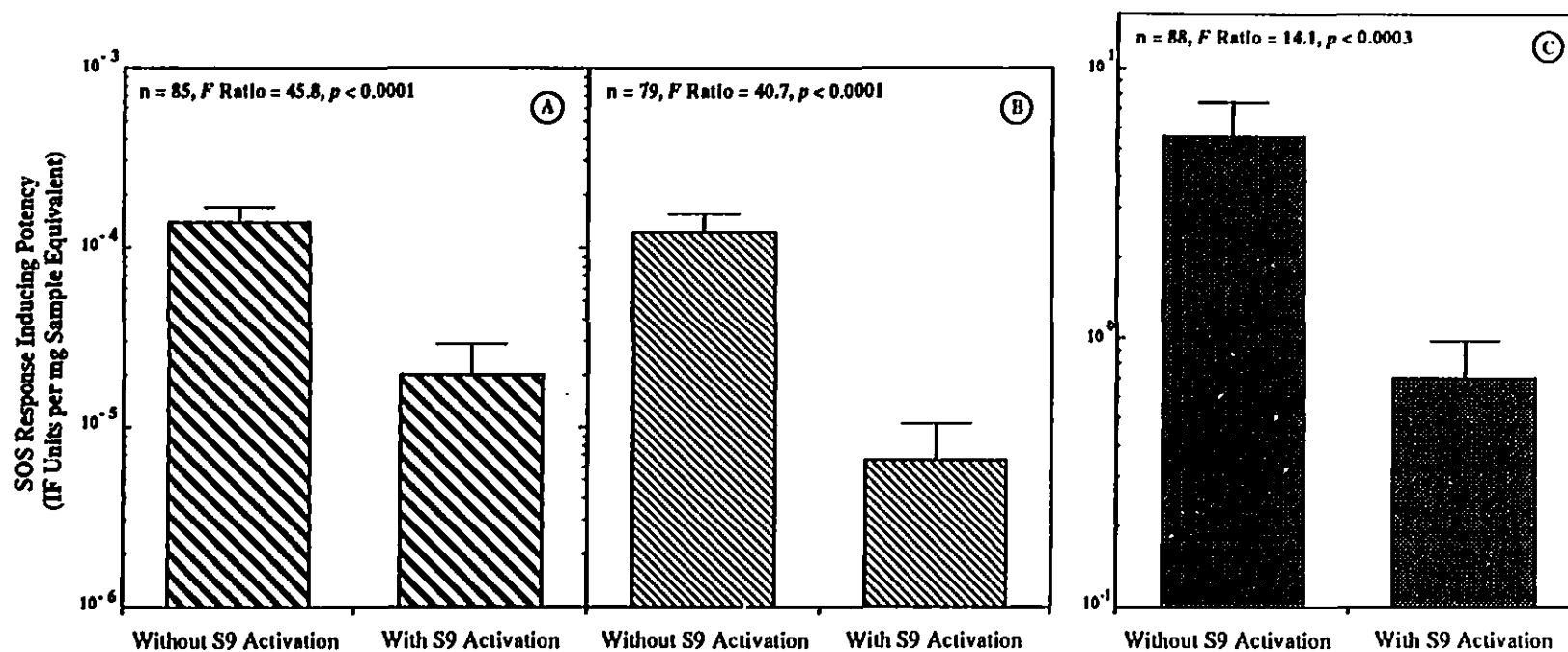
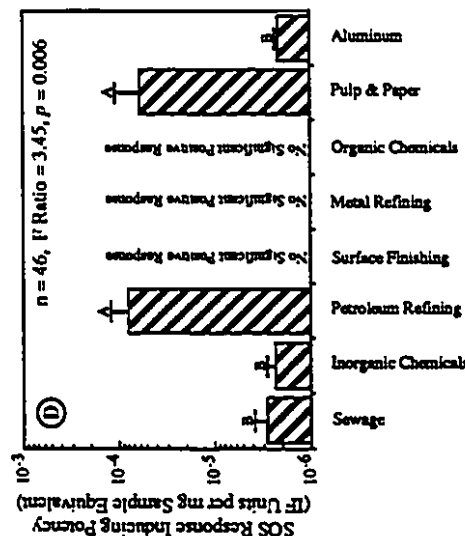
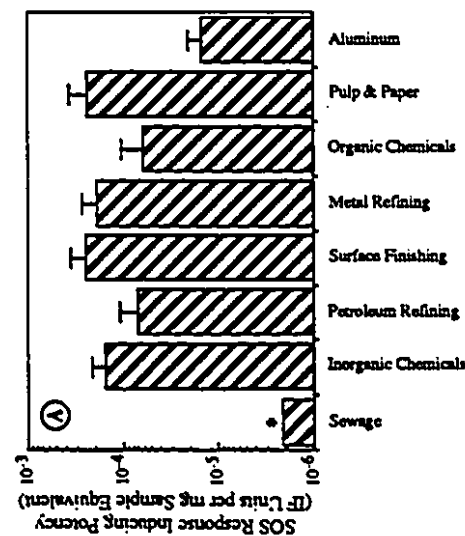
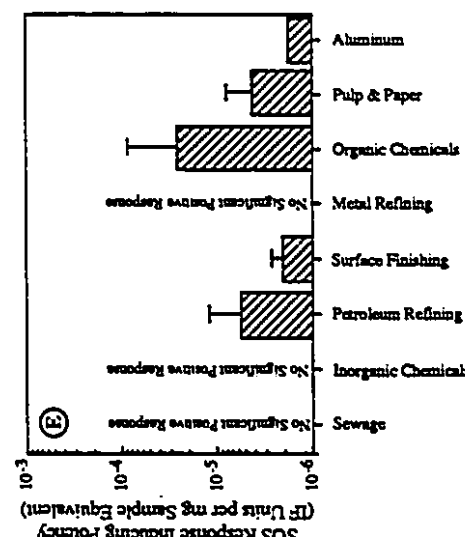
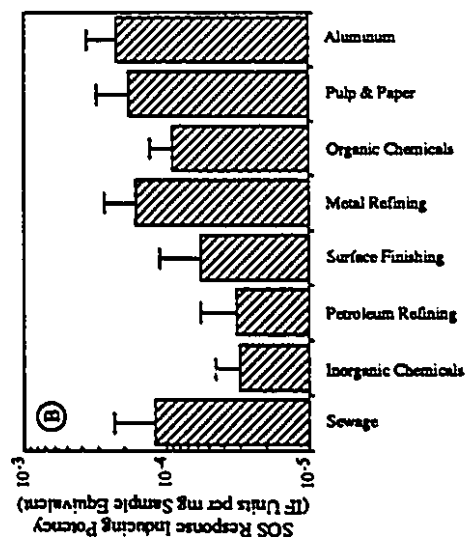
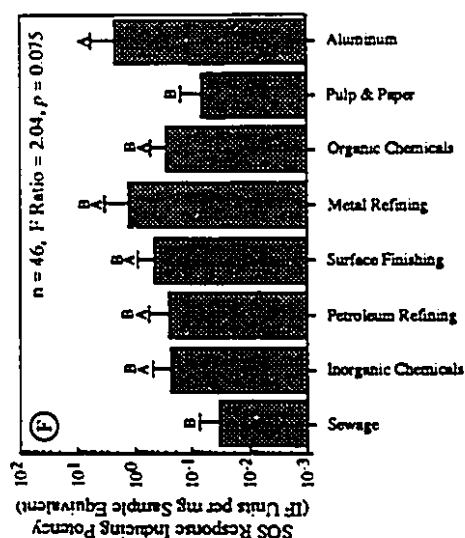
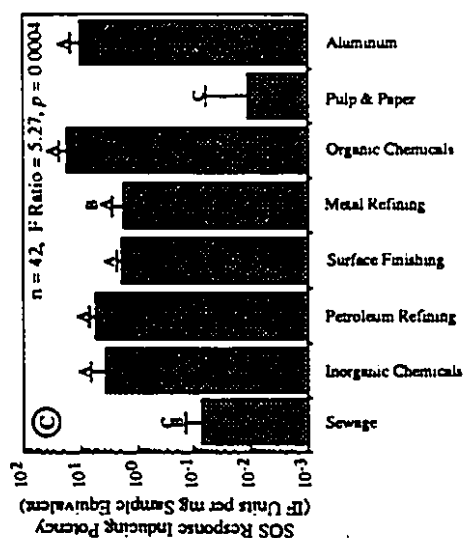


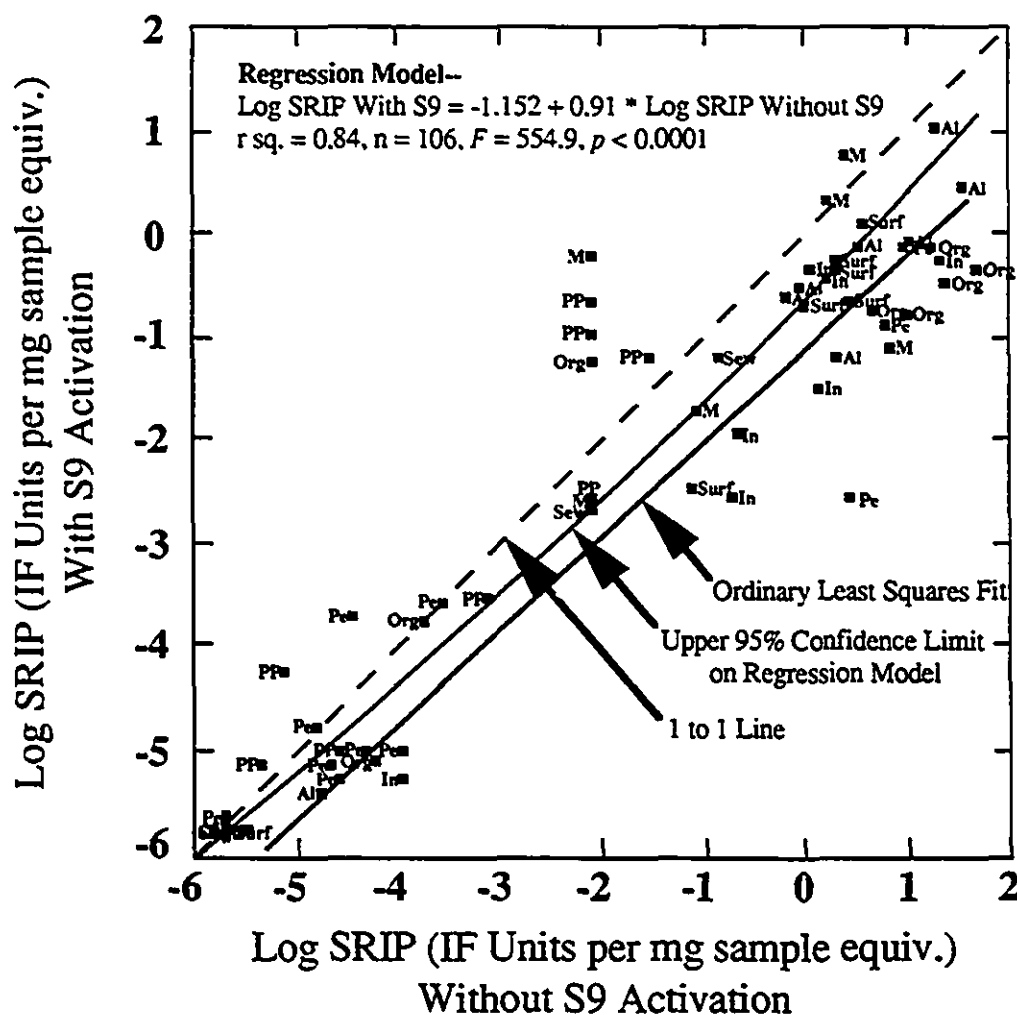
Figure 3. The relationship between industry type and the genotoxic potency (SRIP) of industrial effluent extracts. Mean values for each sample type, both with and without metabolic activation, are shown separately. A- acidic, aqueous extracts without activation; B- alkaline, aqueous extracts without activation; C- suspended particulate extracts without activation; D- acidic, aqueous extracts with activation; E- alkaline, aqueous extracts with activation; F- suspended particulate extracts with activation. Where a significant analysis of variance result was obtained (F Ratio significance < 0.05), the relevant statistics are provided. Bars accompanied by the same letter are not significantly different at $\alpha = 0.05$ (post-hoc comparisons employed the appropriate Bonferonni correction). Error bars are one standard error of the mean. * no standard error available since $n=1$.



refining industries was much less dramatic. The mean aluminum industry SRIP decreased from 9.7 IF Units per mg sample to 2.2 IF Units per mg sample when S9 was added. S9 activation increased the mean genotoxic potency of suspended particulate extracts from three pulp & paper facilities. With respect to aqueous acid extracts, a substantial reduction of genotoxicity in the presence of S9 was observed for samples from surface treatment, organic chemical, inorganic chemical and metal refining facilities. For aqueous alkaline extracts, S9 addition resulted in a substantial reduction in the genotoxicity of samples obtained from metal refining, sewage treatment, inorganic chemical production and pulp & paper facilities. Figure 4 summarizes the relationship between SRIP in the presence of S9 metabolic activation and SRIP in the absence of activation. The figure confirms that the expected potency of an extract tested in the presence of S9 activation is less than that obtained without activation. Several industries lie outside the Bonferonni corrected upper 95% confidence limit (Montgomery and Peck, 1982) of the regression. S9 activation has a minor effect on the potency of extracts obtained from these industries. Only 12 extracts, from 8 industries, elicited a greater response in the presence of the S9 metabolic activation mixture. Seven of the 12 extracts were obtained from suspended particulate samples: 3 from metal refining industries, 3 from pulp & paper facilities and one from an organic chemical production facility. Three aqueous alkaline extracts elicited a greater response in the presence of S9: two petroleum refinery samples and one pulp & paper sample. Two aqueous acid extracts elicited a greater response in the presence of S9: a petroleum refinery sample, and a pulp & paper sample.

Figures 2 and 3 indicate that although industry type and metabolic activation can effect response potency and threshold, sample type (acidic extract, alkaline extract or suspended particulate extract), is the dominant variable. Analysis of variance indicated that sample type can account for 83% of the variation in Log SRIP and 85% of the variation Log GTOX (p values associated with F ratio < 0.0001 . F ratio tests the null hypothesis that all effects in a model are simultaneously zero, and the dependent variable is only a function of its mean and a stochastic disturbance term (Gujarati, 1988)). Analysis of covariance (ANCOVA) revealed that the genotoxic potency (SRIP) and degree of contamination (GTOX) of suspended particulate extracts is related to extract fluorescence, the presence of S9 activation enzymes and the type of industry. Although industry type has a significant effect on both the SRIP and the GTOX of suspended particulate extracts, post-hoc contrasts of regression coefficients (SAS Institute, 1987) revealed that the eight industrial categories listed in Table 1 can be condensed into three groups of industries. The three groups consist of: Group I industries (organic chemical, petroleum refining and aluminum refining)- those that yield highly genotoxic extracts. Group II industries (surface treatment, metal refining

Figure 4. The relationship between the SRIP measured in the presence of S9 metabolic activation and the corresponding potency measured in the absence of activation. Several industries lie outside the upper 95% confidence limit of the regression. Few industries lie above the 1 to 1 line, and are more potent in the presence of S9. The upper confidence limit of the regression was calculated according to Montgomery and Peck (1982). Samples which tested positive in the absence of S9, but failed to elicit a positive response in the presence of S9 are not shown. The following abbreviations are used: Sew-sewage treatment facilities, PP- pulp and paper plants, Al- aluminum refining plants, M- other metal refining plants, Pe- petroleum refining facilities, Surf- surface treatment facilities, In- inorganic chemical production facilities, Org- organic chemical production facilities.



and inorganic chemical)- those that yield moderately genotoxic extracts, and Group III industries (pulp & paper and sewage treatment)- those that yield low potency extracts. Using this industry classification system 46% of the variation in the SRIP of suspended particulate extracts was accounted for by the following ANCOVA model (model (1)) ($r^2 = 0.46$, $n = 74$, F ratio = 14.67, $p < 0.0001$, all effects significant at $p=0.0025$ or less):

$$\text{Log SRIP} = \begin{array}{|l} \text{(A) Group I: } -1.55 \\ \text{(B) Group II: } -1.87 \\ \text{(C) Group III: } -2.47 \end{array} + 0.83 + 0.28 * \text{Log FLUOR} \quad (1)$$

(If S9 Not Present)

Higher level effects were not significant at $p \leq 0.05$. Bold letters indicate the results of multiple comparisons. Coefficients accompanied by the same letter are not significantly different at $p=0.05$. The above ANCOVA result is illustrated in Figure 5a.

The genotoxicity of aqueous extracts is not related to extract fluorescence. However, the results indicate that industry type has a weak effect on the potency of these samples. In addition, S9 activation status has a significant effect on the genotoxicity of aqueous extracts. Figure 5b illustrates the effect of S9 and industry type on the mean SRIP of the aqueous extracts. Post-hoc contrasts of the regression coefficients indicated that the eight industry types can be condensed into two groups: Group I industries (organic chemical, inorganic chemical, metal refining, surface treatment and pulp & paper)- those that yield high potency extracts and Group II industries (sewage treatment, aluminum refining and petroleum refining)- those that yield low potency extracts. In total, industry group and S9 presence can account for only 26% of the variation in the SRIP of the aqueous extracts. The results obtained are summarized in the following ANOVA model (model (2)) ($r^2 = 0.26$, $n = 74$, F Ratio = 12.21, $p < 0.0001$, all effects significant at $p=0.025$ or lower)-

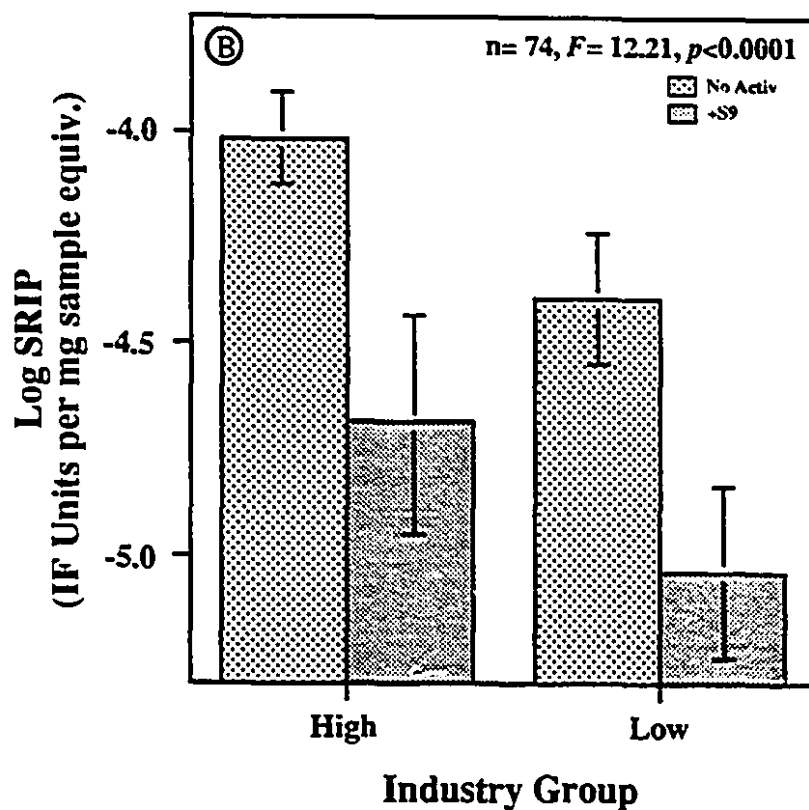
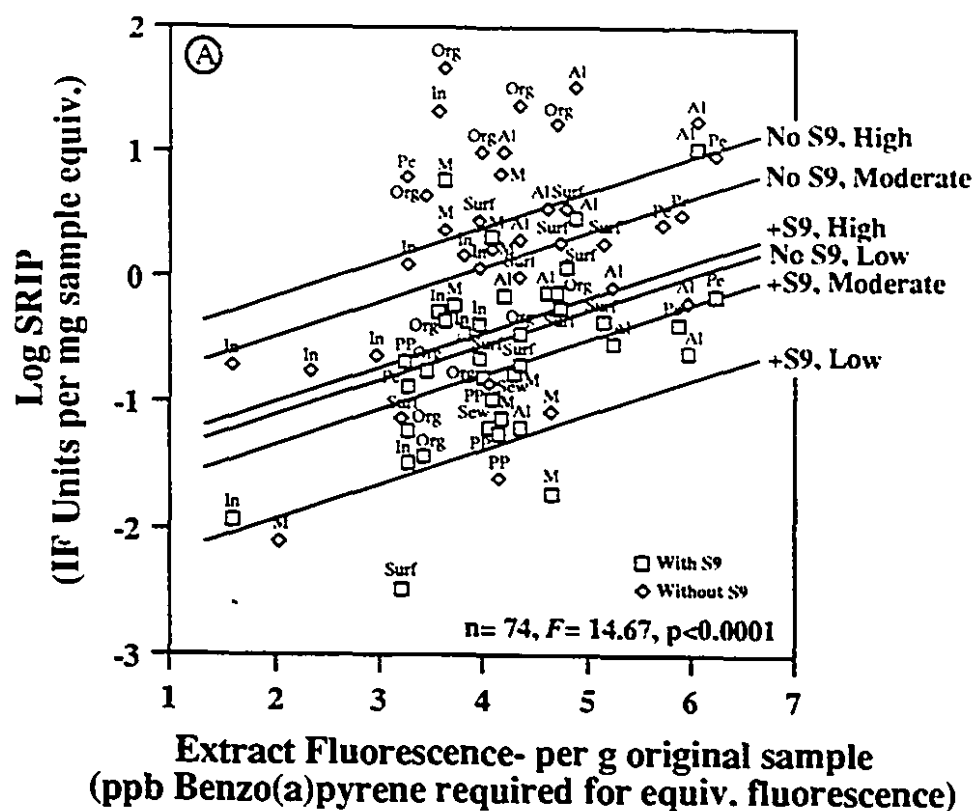
$$\text{Log SRIP} = \begin{array}{|l} \text{(A) Group I: } -4.68 \\ \text{(B) Group II: } -5.04 \end{array} + 0.66 \quad (2)$$

(If S9 Not Present)

Higher level effects were not significant at $p \leq 0.05$. Again, bold letters indicate the results of multiple comparisons.

Genotoxic potency (SRIP) values used to generate Figures 2, 3 and 4 were expressed as IF units per equivalent weight (mg) of the original effluent sample, i.e. suspended particulate matter or aqueous filtrate. Expression of potency per unit of original sample permits a direct comparison of the samples examined, and an assessment of the partitioning of genotoxins between the dissolved and the particle-associated phases (see Fig. 2).

Figure 5. The effect of industry group, S9 presence and extract fluorescence on genotoxic potency. In both plots the dependent variable is SRIP in IF units per equivalent mg of original sample. **A-**The effect of extract fluorescence, S9 presence and industry group on the genotoxic potency of suspended particulate extracts. The fitted lines represent the ANCOVA model (model (1)) described in the text. High potency (Group I) industries include organic chemical, petroleum refining and aluminum production facilities. Moderate potency (Group II) industries include surface treatment, metal refining and inorganic chemical facilities. Low Potency (Group III) industries include pulp & paper and sewage treatment facilities. The difference in potency across industry group and activation status is represented by differences in intercept. **B-** The effect of S9 presence and industry group on the genotoxic potency of aqueous filtrate extracts. Differences in mean genotoxic potency were significant at $p < 0.025$ or lower. Predicted mean values for each condition can be calculated using the model (model (2)) presented in the text. Group I (high potency) industries are organic chemical, inorganic chemical, metal refining, surface treatment and pulp & paper facilities. Group II (low potency) industries are sewage treatment, aluminum refining and petroleum refining facilities. Error bars are one standard error of the mean.



However, some researchers prefer to express potency as response per unit of extractable matter (see review by Houk, 1992). Potency per unit of extractable matter (RES-SRIP) was calculated by dividing potency expressed per unit of original sample by the quantity of extractable matter per unit of original sample. Analysis of covariance (ANCOVA) revealed that the potency of the extractable residues from suspended particulates is also related to extract fluorescence, metabolic activation status and industry type. Again, post-hoc contrasts of the regression coefficients indicated that the eight industry categories listed in Table 1 can be condensed into a smaller number of categories. The resulting industry categories include- high potency (Group I) industries (inorganic chemical and organic chemical production), moderate potency (Group II) industries (aluminum refining, petroleum refining and surface treatment) and low potency (Group III) Industries (metal refining, pulp & paper and sewage treatment). The results obtained indicate that 53% of the variation in RES-SRIP of suspended particulate extracts can be accounted for by extract fluorescence, industry category and metabolic activation status. The ANCOVA results obtained are summarized below in model (3) ($r^2 = 0.53$, F Ratio = 19.81, $p > 0.0001$, all effects significant at $p = 0.05$ or lower)-

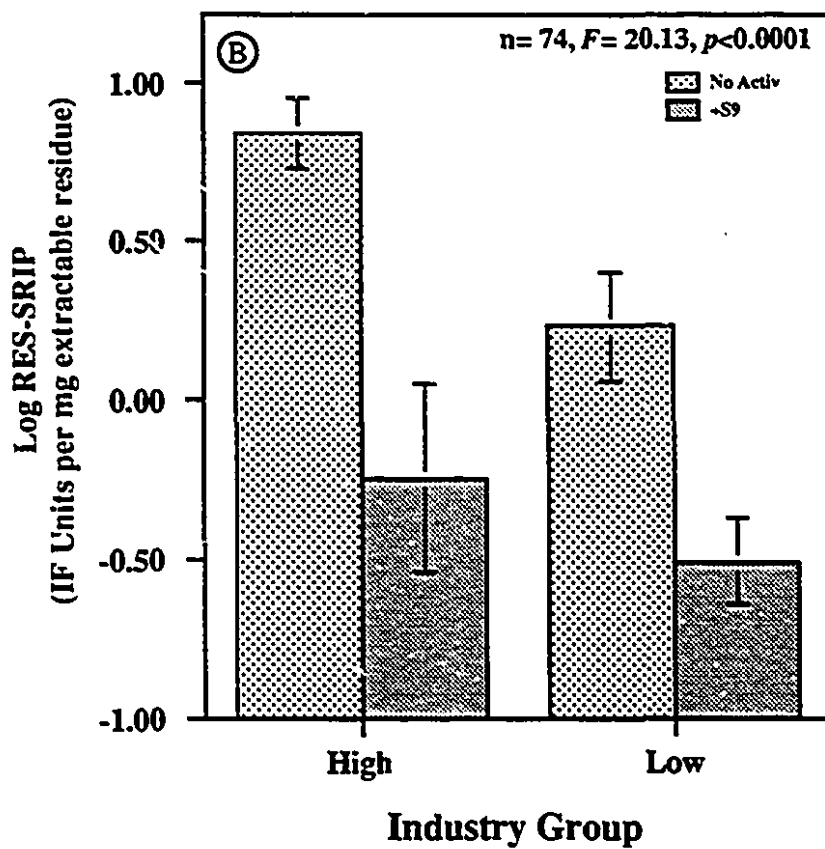
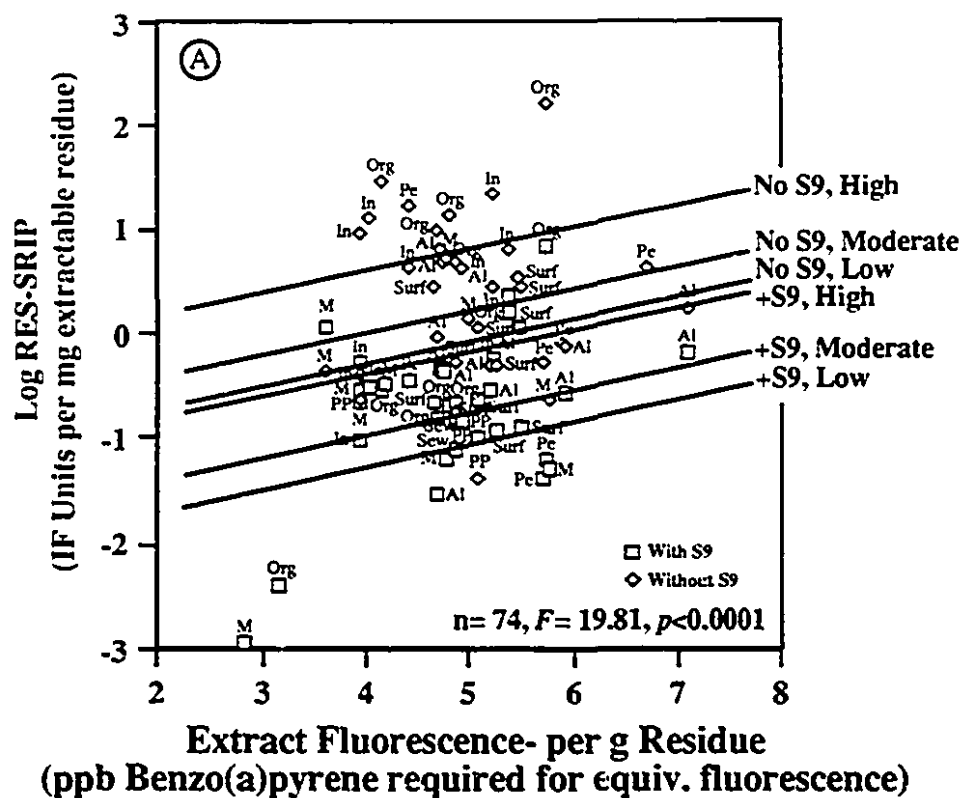
$$\text{Log RES-SRIP} = \begin{array}{|l|} \hline \text{(A) Group I: -1.23} \\ \text{(B) Group II: -1.84} \\ \text{(B) Group III: -2.12} \\ \hline \end{array} + 0.98 + 0.21 * \text{Log FLUOR} \quad (3)$$

(If S9 Not Present)

This model is illustrated in Figure 6a. Higher level effects were not significant at $p \leq 0.05$. Again, bold letters indicate the results of multiple comparisons.

The genotoxic potency of the aqueous extract residues are also related to industry type and S9 activation status. Figure 6b illustrates the effect of S9 and industry type on the mean RES-SRIP of aqueous extracts. Post-hoc contrasts of the regression coefficients indicated that the eight industry types can be condensed into the following two groups: High potency (Group I) industries (inorganic chemical, organic chemical, metal refining, surface treatment and sewage treatment) and low potency (Group II) industries (aluminum refining, pulp & paper and petroleum refining). In total, industry group and S9 presence can account 36% of the variation in RES-SRIP of the aqueous extracts. The results obtained are summarized in the following ANOVA model (model (4)) ($r^2 = 0.36$, $n = 74$, F Ratio = 20.13, $p < 0.0001$, all effects significant at $p = 0.002$ or lower)-

Figure 6. The effect of industry group, S9 presence and extract fluorescence on genotoxic potency expressed per mg of dichloromethane extractable residue. **A-** The effect of extract fluorescence, S9 activation and industry type on the potency of suspended particulate matter extracts. Industry groups are as follows - Group I (high potency industries) are inorganic and organic chemical production facilities, Group II (moderate potency industries) are aluminum refining, petroleum refining and surface treatment facilities, Group III (low potency industries) are metal refining, pulp & paper and sewage treatment facilities. Differences in potency due to industry type and activation status are indicated by differences in intercept. **B-** The effect of S9 presence and industry group on the genotoxic potency of aqueous filtrate extracts. Differences in mean genotoxic potency are significant at $p < 0.002$ or lower. Mean values for each condition can be calculated using the model (model (4)) presented in the text. Error bars are one standard error of the mean. High potency (Group I) industries include inorganic chemical, organic chemical, metal refining, surface treatment and sewage treatment facilities. Low potency (Group II) industries include aluminum refining, petroleum refining and pulp & paper. Error bars are one standard error of the mean.



$$\text{Log RES-SRIP} = \begin{array}{|l} \text{(A) Group I: -0.05} \\ \text{(B) Group II: -0.58} \end{array} + 0.85 \quad (\text{If S9 Not Present}) \quad (4)$$

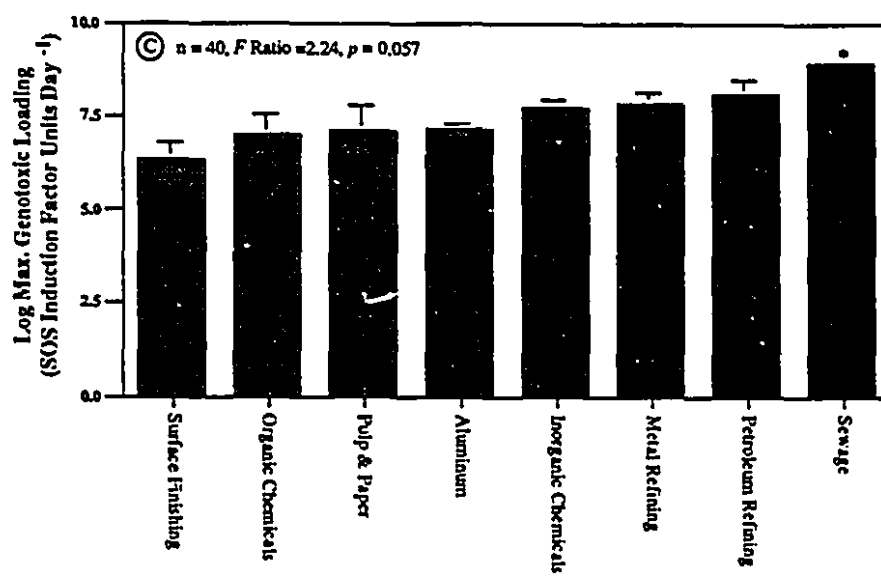
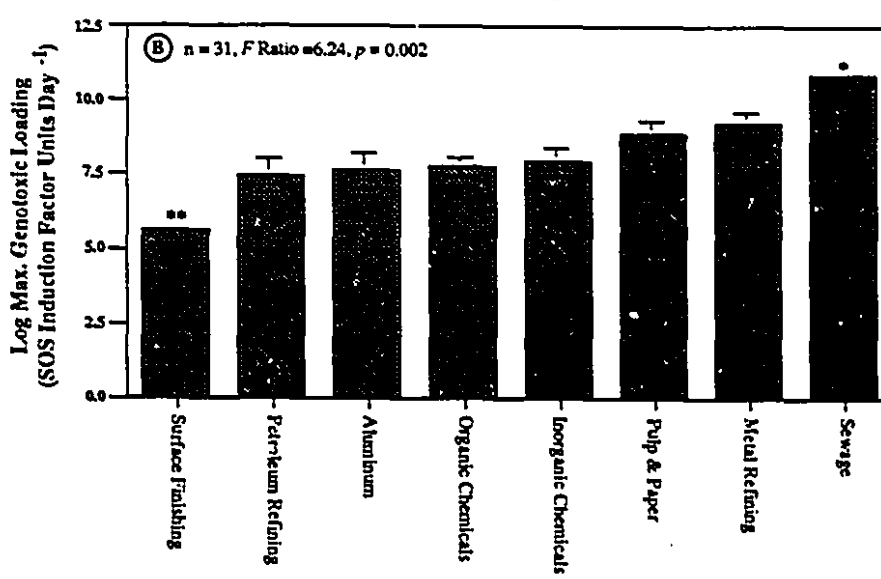
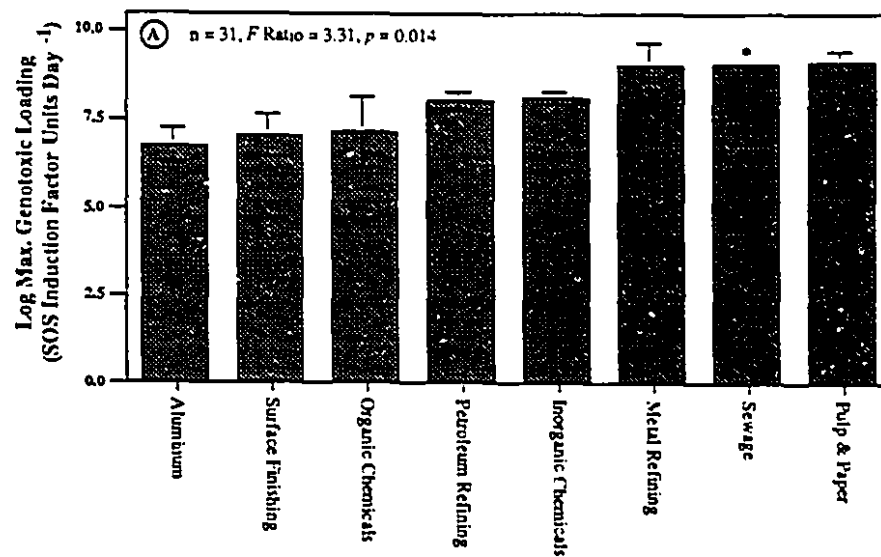
The above model is illustrated in Figure 6b. Higher level effects were not significant at $p \leq 0.05$. Again bold letters indicate the results of multiple comparisons.

In order to compare the genotoxicity of a complex mixture to that of a highly potent pure substance, Metcalfe et al. (1984) converted the Ames mutagenic potency of three effluent extracts to the equivalent mutagenic quantity (μg) of benzo(a)pyrene (BaP) per mg of extractable residue (i.e. the quantity of BaP in μg required to elicit the same response as a mg of extracted residue). They determined that the mutagenic activity of the extractable material from three petroleum refinery particulate matter samples was between 1.2 and 2.4 μg of BaP per mg of residue. We have used the SOS genotoxic potency of BaP (2.52 IF Units per μg^1 - standardized to 1 mL assay volume) to convert RES-SRIP values to their BaP genotoxic equivalents. The mean RES-SRIP of the six petroleum refinery particulate matter samples examined in this study is equivalent to 2.3 μg of BaP per mg of residue. The highest RES-SRIP value obtained corresponds to the particulate matter extract from organic chemical facility #2. Residue potency for this sample is the equivalent of 63 μg of benzo(a)pyrene per mg of residue.

Figures 3, 5 and 6 reveal large differences in potency across industry type. However, they do not indicate differences in genotoxic loading. The hazard posed to the aquatic ecosystem by a given discharge depends not only on the potency of the waste stream, but on the mean daily discharge of the contaminated stream. Daily genotoxic loading values (IF per day) were calculated from genotoxic potency (IF per equivalent unit of effluent), suspended particulate concentration (mg per L) and daily discharge values (L per day). For aqueous filtrate samples, daily loading is calculated as the product of the SRIP (converted to IF per equivalent liter of sample) and the mean daily discharge in liters per day. For suspended solid samples, potency values were first converted to IF per equivalent liter by multiplying the SRIP values by the TSP in mg per liter. The results obtained are summarized in Figure 7. The values presented are based on the maximum genotoxic loading values, calculated using the activation status that produced the highest response. ANOVA revealed a significant industry type effect on the daily genotoxic loadings of both acid and alkaline aqueous extracts. Industry type does not have a significant effect on the daily genotoxic loading from suspended particulate matter. The figure clearly demonstrates that sewage treatment effluents, which provided low potency extracts (see Figure 3), have very high genotoxic loadings. On the contrary aluminum refinery effluents, which

¹mean of 40 separate measurements made in our laboratory.

Figure 7. Mean, maximum genotoxic loading values for the eight industry types examined. **A-** Genotoxic loadings from acidic constituents of industrial effluents. ANOVA revealed a significant industry type effect ($r^2 = 0.50$, $n = 31$, F ratio = 3.31, $p = 0.014$). **B-** Genotoxic loadings from alkaline constituents of industrial effluents. ANOVA revealed a significant industry type effect ($r^2 = 0.77$, $n = 21$, F ratio = 6.24, $p = 0.002$). **C-** Genotoxic loadings from effluent suspended particulate matter. ANOVA did not reveal ($\alpha = 0.05$) a significant industry type effect ($r^2 = 0.33$, $n = 40$, F ratio = 2.24, $p = 0.057$). Error bars are all one standard error of the mean. * - no standard error was calculated since $n=1$. ** - error bar too small to be shown.



frequently provided highly potent extracts, have substantially lower loading than sewage treatment effluents. The mean genotoxic loading of suspended particulates from organic chemical production facilities is significantly lower than sewage treatment loading values, despite the fact that organic chemical effluent samples provided the most potent suspended solid extracts.

The genotoxic potency of any well known genotoxin can be used to convert the loading values presented in Figure 7 to a more meaningful chemical equivalent. Again, the SRIP of BaP can be used. Using the aforementioned BaP SRIP value, a loading of 2.52×10^9 SOS IF Units per day corresponds to a genotoxic equivalent of 1 kg of benzo(a)pyrene per day. Employing this conversion, genotoxic loadings of suspended particulates ranged from 0.032 g BaP equivalents per day for surface treatment industry #5 to 547 g BaP equivalents per day for metal refining facility #6. Genotoxic loadings from aqueous, acidic compounds ranged from 0.05 g BaP equivalents per day for organic chemical industry #3 to 7429 g BaP equivalents per day for metal refinery #5. Genotoxic loadings from aqueous, alkaline compounds ranged from 0.2 g BaP equivalents per day for surface treatment industry #5 to 30262 g BaP equivalents per day for sewage treatment facility #2. Minimum, maximum and geometric mean loading values in BaP genotoxic equivalents are summarized in Table 3.

Empirical models that relate published Ames mutagenic potency values to published SOS response genotoxic potency values (White and Rasmussen, 1995) were used to predict the mean mutagenic potency (revertents per mg extractable residue) of suspended particulate and aqueous filtrate samples. The models employed for the conversion describe the relationship between SOS genotoxic potency and Ames mutagenic potency across 254 direct acting genotoxins and 118 S9 mediated genotoxins. Many suspended particulate, aqueous acid and aqueous alkaline extracts yielded predicted mutagenic potency values between 10^3 and 10^4 revertents per mg residue, with maximum values approaching 2×10^5 revertents per mg residue. Geometric mean values are between 10^4 and 10^6 revertents per mg. Minimum, maximum and geometric mean mutagenic potency values for each sample type and activation condition are presented in Table 4. The values are provided solely to permit a comparison of the results obtained in this study with the large body of published Ames test results (see review by Houk, 1992).

Table 3. Daily genotoxic loadings expressed as g benzo(a)pyrene genotoxic equivalents. Loading values in SOS induction factor units per day were converted to an equivalent (genotoxic) quantity of benzo(a)pyrene (BaP) using the mean SOS genotoxic potency of BaP.

Industry Type	Minimum	Maximum	Geometric Mean
Suspended Solid Extracts			
Surface treatment	0.032	37.88	0.91
Inorganic chemical prod.	9.16	98.11	23.53
Aluminum refining	0.99	23.62	5.39
Organic chemical prod.	0.059	261.54	4.01
Petroleum refining	10.23	306.26	52.36
Non-Al metal refining	2.04	546.90	27.61
Sewage treatment	348.47	348.47	348.47 [†]
Pulp and paper	0.31	60.27	5.29
Acidic Filtrate Extracts			
Surface treatment	0.36	100.00	4.51
Inorganic chemical prod.	16.96	93.33	53.16
Aluminum refining	0.29	18.17	2.32
Organic chemical prod.	0.052	76.61	5.60
Petroleum refining	19.57	396.95	45.32
Non-Al metal refining	1.92	7428.57	450.32
Sewage treatment	496.58	496.58	496.58 [†]
Pulp and paper	98.55	4686.72	568.18
Alkaline Filtrate Extract			
Surface treatment	0.12	0.21	0.16
Inorganic chemical prod.	11.26	92.74	32.32
Aluminum refining	1.15	109.58	16.31
Organic chemical prod.	4.27	96.96	22.45

Table 3 continued.....

Petroleum refining	2.77	155.07	4.90
Non-Al metal refining	312.38	1672.20	722.75
Sewage treatment	30261.94	30261.94	30261.94 [†]
Pulp and paper	23.52	2674.77	292.77

[†] Significant positive response obtained for a single sample only.

Table 4. Predicted mean Ames mutagenic potency values (revertents per mg of residue). All predicted potency values were rounded to the nearest whole number. Mutagenic potency values were predicted from the observed SRIP data using empirical models based on literature data (White and Rasmussen, 1995). Models employed predict the mean, maximum Ames mutagenic potency. For direct acting samples the model used is- Log Max. Mutagenic potency (Revertents per μg - normalized to top agar volume of 1mL) = $2.28 + 0.78 * (\text{Log SOS response inducing potency in IF Units per } \mu\text{g- normalized to an assay volume of 1mL})$ -- $r^2 = 0.69$, $n = 254$, $F = 551.7$. For S9 mediated samples the model used is- Log Max. Mutagenic potency (Revertents per μg - normalized to a top agar volume of 1mL) = $3.04 + 0.94 * (\text{Log SOS response inducing potency in IF Units per } \mu\text{g- normalized to an assay volume of 1mL})$ -- $r^2 = 0.71$, $n = 118$, $F = 282.3$. Predictions of mutagenic potency converted back to arithmetic scale were multiplied by a correction factor to account for the inherent bias in log transformed equations. Correction factors were calculated according to Neyman and Scott (1960)-- $\text{CF} = \text{antilog}(1.1513 \times \text{RMS})$ where RMS is the residual mean square of the regression model.

Sample	Minimum	Maximum	Geometric Mean
Suspended Particulates without S9	294	1.8×10^5	6508
Suspended Particulates with S9	9	3.1×10^4	1135
Aqueous Acid without S9	588	5.9×10^4	8600
Aqueous Acid with S9	452	1.1×10^4	2682
Aqueous Alkaline without S9	406	1.3×10^5	9949
Aqueous Alkaline with S9	263	3.3×10^4	1295

DISCUSSION

The goal of this work was to investigate the presence, potency and potential hazard of genotoxins in a wide variety of industrial effluents, representing a wide range of industrial processes. Before proceeding with a detailed discussion of the results, it is appropriate to acknowledge some of the more serious limitations of the data. Although a large number of samples were tested, genotoxicity assessment was performed using a single, short-term assay that employs a single organism. Several researchers (e.g. Quillardet and Hofnung, 1988; Brusick et al., 1992) have discussed the merits of using several assays with multiple endpoints for genotoxicity assessment. In addition, it should be noted that the SOS Chromotest is not well-suited for the detection of alkylating agents (e.g. methane and ethane sulfonic acid esters) and base analogues (e.g. 2-aminopurine and 5-bromouracil) that do not normally induce the SOS, error-prone pathway (Quillardet and Hofnung, 1993).

While the fluorescence spectrophotometry measurements provide a non-specific measure of extract contamination, the values obtained only provide a semi-quantitative indication of the concentration of aromatic substances in the extracts. The two main problems with this method of analysis are: 1) substances that do not fluoresce at 430nm cannot be detected, and 2) according to Beer's law the total extract fluorescence will be dependent on the molar fluorescence of each mixture component in addition to their individual concentrations. However, it is interesting to note that many 5- and 6-ring polycyclic aromatic hydrocarbons have similar molar absorptivities at 380nm (Friedel and Orchin, 1951). These and closely related substances likely make a large contribution to the measured fluorescence values.

A third limitation stems from the losses of volatile substances during the extraction and solvent exchange procedures. All extracts were taken to dryness in order to exchange dichloromethane for dimethyl sulfoxide. Although the lower molecular weight, more volatile aromatic hydrocarbons (e.g. naphthalene) are not usually considered a high priority in genotoxicity research, these and similar substances may have been lost during solvent exchange. Spiking experiments confirmed such losses and indicate that $\geq 50\%$ of 2- and 3-ring polycyclic aromatics can be lost during chemical work-up and solvent exchange. In addition, dichloromethane's solvation properties may limit its ability to recovery higher molecular weight aromatic hydrocarbons from aqueous samples.

The presence of an S9 metabolic activation mixture had a significant effect on extract genotoxicity. S9 microsomal enzymes were added to the assay mixture to permit the detection of substances that are pro-genotoxic and must be converted to an active genotoxin

during exposure. However, for the majority of extracts, the presence of S9 enzymes resulted in a reduction of extract genotoxicity. Similar effects of S9 enzymes on industrial wastes and effluents have been documented. Hopke et al. (1984) tested extracts of municipal wastewater sludge and found that S9 reduced the mutagenic potency of all but one sample. Bjørseth et al. (1979), McGeorge et al. (1985), Nestmann et al. (1979) and Kamra et al. (1983) found that the mutagenicity of pulp & paper mill effluent extracts is dramatically reduced by S9 enzymes. Several similar examples are available that describe the effects of S9 on an organic chemical manufacturing effluent, a textile dyeing effluent (McGeorge et al., 1985) and a pharmaceutical manufacturing waste (Houk and deMarini, 1988). The cause of the genotoxicity reduction is not clear. It may be due to enzyme catalyzed detoxification. However, Kamra et al. (1983) have shown that S9 activation mixtures that do not contain the required cofactors or contain heat inactivated S9 enzymes are also capable of reducing the mutagenicity of industrial wastes.

In several cases the presence of S9 had a very minor effect on genotoxicity or even increased the response. An increase in genotoxicity in the presence of S9 is common for pure compounds and defines promutagenicity, whereby compounds must be oxidized to a DNA damaging electrophilic species (Heflich, 1991). An increase in potency in the presence of S9 (see Fig. 4) was observed for 12 extracts from metal refining, pulp & paper, organic chemical and petroleum refining facilities. Both McGeorge et al. (1985) and Somani et al. (1980) demonstrated that foundry effluent samples require S9 activation. Houk (1992) cited several studies that measured the Ames mutagenicity of organic chemical production effluents and concluded that S9 often converts the samples into more potent mixtures. McGeorge et al. (1985) and Metcalfe et al. (1985) demonstrated that extracts of petroleum refinery effluents can be more potent in the presence of S9. The observed S9 effect on sewage treatment effluents was very minor. While Hopke et al. (1984) demonstrated that S9 can decrease the mutagenicity of municipal wastewater sludge, several researchers (Waters et al., 1989; Meier et al., 1987; Brown and Donnelly, 1988) report an increase in mutagenicity in the presence of S9. Five of the 12 samples that elicited a greater response with S9 were obtained from pulp & paper mills. This is interesting, given the aforementioned negative effect of S9 on the Ames mutagenic potency of pulp & paper effluent samples. Extracts that had an equal or higher potency when tested in the presence of S9 likely contain some compounds that are not genotoxic *per se*, but require metabolic activation. A large number of genetic toxins including nitrosamines, carbamates, arylamines, arylamides, heterocyclic amines and polycyclic aromatic hydrocarbons (PAHs) require metabolic activation (Heflich, 1991). Petroleum refineries, foundries, aluminum

refineries and sewage treatment facilities are all well known for their discharge of PAHs and related compounds (IARC, 1989a; IARC, 1984; Woo and Arcos, 1981).

Sample type had a large effect on both extract potency and contamination. In several cases large differences exist between the genotoxicity of aqueous acid samples and the corresponding alkaline sample. While both samples can contain neutral compounds, only the acid extract can contain organic acids, phenols and cresols. Only the alkaline fraction can contain arylamines and heterocyclic amines (Alfheim et al., 1984). Although differences in potency may result from differential destruction or chemical alteration of various chemical constituents (Tabor et al., 1985), comparisons of alkaline and acid extracts can yield insight into the chemical nature of the putative genotoxins. Despite previous investigations of surface finishing industries which failed to find Ames mutagenicity in effluent extracts (McGeorge et al., 1985), the samples studied here provided the most potent aqueous acid samples. This may indicate contamination with cresols and other phenolics used in degreasing, machining and metal coating (Environment Canada, 1987). High potency aqueous acid extracts were also obtained from pulp & paper effluent samples. Several researchers have determined that resin acids and chlorinated phenolics (primarily catechols and guaiacols) present in pulp mill effluents account for most of the mutagenicity (Nestmann et al., 1979; Holmbom et al., 1984; Kringstad and Lindstrom, 1984). Aqueous alkaline extracts from pulp and paper mills were also quite potent. This may indicate the presence of neutral genotoxins such as chlorinated ketones and chlorinated aliphatics (Houk, 1992; Douglas et al., 1983). Alkaline extracts of sewage treatment effluents were found to be more potent than their corresponding acidic extracts. Similar results were obtained by Rappaport et al. (1979). Detailed chemical analyses of mutagenic samples from a municipal wastewater effluent identified several aromatic amines including the known mutagen *N*-nitrosodimethylamine (Ellis et al., 1982). Acid extracts from petroleum refinery effluents were found to be more genotoxic than their alkaline counterpart. This suggests that the putative genotoxins are primarily neutral and acidic compounds. Several researchers (Epler et al., 1978; Wilson et al., 1980; Metcalfe et al., 1985) have revealed that the neutral fraction of industrial discharges from petroleum related industries contain the putative genotoxins. Epler et al. (1978) also describes fairly potent acid fractions derived from a coal gasification effluent. The genotoxicity of both aqueous extracts from organic chemical production facilities are quite similar. This may indicate that the putative genotoxins are acidic, basic and neutral compounds. Although this industrial sector is the largest producer of hazardous waste (Houk, 1992), it is difficult to compare the results obtained to previously published studies due to the enormous variety of products, manufacturing processes and raw materials. The genotoxicity of the two aqueous

extracts obtained from metal refining facilities are also quite similar. A wide range of neutral PAHs and arylamines have been identified in metal founding effluents and they are thought to be responsible for effluent mutagenicity and carcinogenic hazard (Houk, 1992; IARC, 1984). The alkaline extract from aluminum refining facilities are far more potent than the corresponding acid sample. This suggests that the putative genotoxins may include arylamines as well as the neutral PAHs which are known to be an undesirable by-product of the electrolytic reduction of aluminum (IARC, 1984).

Figure 2 indicates that when the genotoxic potency of suspended particulate extracts is expressed per unit (mg) of starting material, the particulate matter extracts can be as much as five orders of magnitude more potent than their corresponding aqueous samples. This suggests that the putative genotoxic substances have a high tendency to associate with particulate matter. Extract fluorescence measurements suggest that suspended particulate samples are far more contaminated with aromatic substances (10-100 ppm of benzo(a)pyrene required for equivalent fluorescence) than their corresponding aqueous samples (0.1-10 ppb of benzo(a)pyrene required). The industry group assignments shown in Figure 5 permit an initial examination of the sorptive properties of the genotoxins in various industrial effluents. The results indicate that the most potent and contaminated samples of particulate matter were obtained from organic chemical production facilities as well petroleum and aluminum refineries. Pulp & paper and sewage treatment facilities provided the least potent particulate matter samples. Conversely, pulp & paper plants yielded high potency filtrate samples, while petroleum and aluminum refineries provided low potency filtrate samples. This suggests that pulp & paper mills emit mostly soluble genotoxins, while petroleum and aluminum refineries emit genotoxins that are predominantly associated with suspended particulate matter. The sorption of genotoxins and fluorescent substances to effluent suspended particulate matter will be examined and discussed in detail in a companion work (see White et al., 1995b).

In contrast to the results presented in Figure 2, there is very little difference between the potency of the aqueous filtrates and suspended particulate samples when the results are expressed per unit of extractable residue. This is due to the fact that the quantity of extractable material recovered from the particulate matter samples is frequently 4 to 6 orders of magnitude higher than that obtained from the corresponding aqueous filtrate samples. Wide variation in the amount of extractable material per unit of original sample caused the differences in the industry classification systems shown in Figures 5 and 6. For example, aqueous filtrate samples from sewage treatment facilities yielded low potency extracts when the results are expressed per unit of original sample (see Figure 5b). However, these samples yielded very small quantities of extractable residue. The net result is a high potency

value expressed per unit of extractable residue. The results presented in Figure 6 indicate that although the quantity of extractable material obtained from organic and inorganic chemical production facilities is low compared with other samples, the recovered material has a higher genotoxic potency than that recovered from other industries.

With the exception of the works of Houk and Claxton (1986), deMarini et al. (1987), Houk and DeMarini (1988), Somani et al. (1980), Sanchez et al. (1988) and McGeorge et al. (1985) attempts to investigate genotoxic organics in industrial effluents have focussed on one particular industry or one industrial category. Previous works that examined samples from many industries belonging to several different industrial categories were not able to find any statistically significant relationship between genotoxicity (or mutagenicity) and industry type. McGeorge et al. (1985) attributed the lack of a quantitative relationship to low sample number and large variations in production and waste treatment processes. Despite differences in production processes, waste treatment processes and raw material use, we found that industry type had a significant effect on the potency of suspended particulate and aqueous filtrate extracts. However, the eight industry types listed in Table 1 can be condensed into a smaller number (2-3) of industrial categories. The assignment of industries into the various groups depends on the sample type (i.e. filtrate or suspended solids) as well as the unit used for expression of genotoxic potency. Despite differences in industry classification, Figures 5a and 6a indicate that the suspended particulate matter emitted by inorganic and organic chemical production facilities, petroleum and metal refineries as well as surface treatment facilities are significantly more potent than that emitted by sewage treatment and pulp & paper facilities. In addition, Figures 5b and 6b indicate that the aqueous filtrates of inorganic chemical, organic chemical, metal refining and surface treatment facilities are significantly more potent than those from aluminum and petroleum refineries.

Classification of industries according to RES-SRIP (potency per unit extractable residue) permitted a comparison of the results obtained to a recent review of the genotoxicity of industrial wastes (Houk, 1992). Houk (1992) reviewed a large number of published works which assessed the mutagenicity of wastes from pulp & paper mills, foundries, chemical manufacturing facilities and petroleum refineries. The industry classification system presented in Figure 6a corresponds reasonably well to the classification system of Houk (1992). Houk's classification system noted that highly mutagenic effluent samples are frequently obtained from organic chemical and munitions production facilities and moderately mutagenic samples are frequently obtained from petroleum refineries, petrochemical facilities and foundries. Although Houk demonstrated

that the mutagenicity of pulp and paper mill effluents is variable, some whole mill effluent samples were placed in the low mutagenic potency category.

Comparison of the RES-SRIP values to the potency of BaP indicates that a mg of extracted residue is the (SOS) genotoxic equivalent of 0.1 to 1 μg of BaP. Therefore, the complex mixture of dichloromethane extractable material is 3 to 4 orders of magnitude less potent than actual BaP. Although these results do not indicate or suggest that BaP is actually present in the extract they provide an interesting comparison between the genotoxicity of a complex extract and a well-known, potent genotoxin. Determining whether or not BaP itself is actually present in any of the extracts would require chemical-specific analyses.

Figures 5a and 6a indicate that effluent genotoxicity is related to extract fluorescence. The weak effect of fluorescence on potency expressed per unit of extractable residue can be attributed to the lack of variability in fluorescence per unit residue. It is tempting to use the relationship shown in Figure 5a to infer that the putative genotoxicants are polycyclic aromatic substances. Potent genotoxins isolated from a variety of wastes and contaminated environmental samples are often polycyclic aromatic substances (IARC, 1984; West et al., 1988; Grifoll et al., 1990; Balch et al., 1995; Marvin et al., 1993; Woo and Arcos, 1981). Many of these substances, by virtue of containing conjugated chromophores, are capable of fluorescing when excited by radiation in the near UV range (Cheng and Young, 1986; Friedel and Orchin, 1951). However, the fluorescence measurements do not permit a differentiation between (homo)polycyclic aromatics or any other fluorescent component of the mixture. Homocyclic PAH derivatives, as well as heterocyclic aromatics and their derivatives, likely contribute to the total fluorescence and may be responsible for the genotoxicity. Recent publications describe the use of bioassay directed fractionation to isolate a variety of homocyclic PAHs (Marvin et al., 1993), homocyclic PAH derivatives (Griffol et al., 1990; Saleem et al., 1984; Schleibinger et al., 1989) as well as sulfur and nitrogen heterocyclics from mutagenic complex extracts (West et al., 1988; Balch et al., 1995).

Use of empirical models to predict the mean mutagenic potency of the samples examined provides a broader framework within which the results obtained can be evaluated. Predicted Ames mutagenic potency values are similar to previously published values. Most of the waste samples reviewed by Houk (1992) were found to have mutagenic potencies between 10^2 and 10^5 revertents per mg extract. The majority of samples examined in this study have predicted mutagenic potency values that correspond to Houk's low or moderate potency category. The highest predicted mutagenic potency values (in the 10^5 revertents per mg extractable matter range) would be categorized by Houk as

high potency wastes. None of the samples examined yielded predicted mutagenic potencies as high as the extremely mutagenic petrochemical waste oil and plastic tar described by Houk. It should be noted that the data used to construct the empirical models employed to predict maximum mutagenic potency do not include many measurements of complex mixtures. However, the results presented by White and Rasmussen (1995) indicate that the residual variation in the predicted mutagenic potency of a complex mixture is often smaller than the mean residual variation (i.e. the root mean square error of the model).

We have demonstrated that industrial and sewage treatment discharges are clearly genotoxic on the SOS Chromotest. Up to 70% of the extracts tested, depending on the sample type and the activation status, elicited a positive response. Other multi-industry studies that used the Ames test rarely obtained positive responses in more than 50% of the samples. McGeorge et al. (1985) examined effluents from 27 industries. Only 9 samples elicited a clear positive response. Sanchez et al. (1988) examined effluents from 75 industries. Only 22 samples elicited a clear positive response. The number of positive responses obtained in this study may not be surprising considering that most of the industries examined were previously recognized for the toxicity of their liquid discharges (GIPASL, 1992). Alternatively, the preponderance of positive responses may confirm the sensitivity of the SOS response as an indicator of complex waste genotoxicity. A number of recent works have commented on the use of the SOS response as an indicator of genotoxic stress. The response pathway appears to be a useful bioassay endpoint since it can be invoked by a wide range of DNA damage events (Elespuru, 1987; Hofnung and Quillardet, 1986; Rossman et al., 1985; Houk and deMarini, 1988). Since few researchers have used the SOS Chromotest or other DNA damage/repair assays to study complex waste samples, the present work substantially increases the available information on the ability of complex environmental extracts to induce the SOS response pathway of *E. coli*. Despite its *relative* lack of use, the SOS Chromotest has several attributes that make it an attractive bioassay system. These include: 1) short incubation time (results obtained in a single working day), 2) assay easily carried out in microtiter plates, 3) method automation accomplished using readily available laboratory equipment, 4) survival of tester strain is not required, 5) sample sterility is not required, 6) sample toxicity (growth inhibition) is simultaneously monitored, 7) the response pathway is induced by a wide range of DNA damage events (Elespuru, 1987; Hofnung and Quillardet, 1986; Rossman et al., 1985; Houk and deMarini, 1988) and 8) the assay can easily accept biological samples such as tissue and excrement extracts which may contain nutrients that can impair the performance of other assay organisms (Parry, 1985).

Variation in potency and contamination, while interesting, do not give any indication of the potential impact of a given effluent on the receiving water. Extremely high daily loading values were calculated for several industry types including petroleum refineries, pulp & paper mills, metal refining facilities and sewage treatment plants. Using the published genotoxic potency of the carcinogen BaP, the daily loading values were converted to BaP genotoxic equivalents in grams per day. The results indicate that few industries release more than the SOS genotoxic equivalent of 1 kg of BaP per day. Only Sewage treatment facility #2 releases more than the genotoxic equivalent of 10 kg of BaP per day. Metcalfe et al. (1985) performed a similar calculation and determined that the suspended particulate matter emitted by a petroleum refinery is the mutagenic equivalent of 1.9 kg benzo(a)pyrene per day. Loading values for the suspended particulate samples of the petroleum refineries examined in this study ranged from 10g BaP equivalent per day for petroleum refinery # 2 to 306 g BaP equivalent per day for petroleum refinery # 4. The 2.5-fold difference in discharge between petroleum refinery #4 and the refinery studied by Metcalfe et al. cannot account for the 6-fold difference in daily loading rates. It is interesting to note that the daily, total genotoxic loading from Organic chemical industry #3 is slightly greater than the equivalent of 0.1 g of BaP. Despite using creosote preparations that are known to contain genotoxic PAHs (Environment Canada, 1988b), and an annual production of 2500 m³ of creosote treated wood, an extensive water reuse and treatment system manages to reduce the daily genotoxic loading to a level that is barely detectable (based on a 4 Liter 24 hour integrated sample).

The aforementioned loading values suggest that the potential hazard of the discharges examined is likely substantial. Actual hazard and relative risk assessment is complicated by our poor understanding of the post-emission behavior of toxicants, the subsequent downstream exposure levels and the *in situ* effects of this exposure. Nevertheless, several researchers have detected genotoxins in surface waters (Maruoka et al., 1986; Sloof and Van Kreijl, 1982; Langevin et al., 1992; Van Hoof and Verheyden, 1981) as well as bottom sediments (Grifoll et al., 1990; Sato et al., 1983; Metcalfe et al., 1990; Durant et al., 1990). In addition, genotoxic, mutagenic and clastogenic effects on aquatic plants (Klekowski and Levin, 1979; Klekowski and Berger, 1976; Ravindran and Ravindran, 1978), fish (diGiulio et al., 1993; Prein et al., 1978; Alink et al., 1980) and bivalves (Dixon, 1982; Rodriguez-Ariza et al., 1992) exposed to water contaminated by large industries have been described. However, the evidence linking industrial loading to post-emission, genotoxic effects is still circumstantial. Future research must examine the post-emission exposure and effect of industrial genotoxins in an effort to determine the long term prospects for communities of aquatic biota continually exposed to industrial wastes. In

addition, the use of bioassay-directed fractionation to isolate and identify the putative genotoxins in the samples investigated seems a promising area for future research.

ACKNOWLEDGMENTS

Special thanks to the personnel at Environment Canada who provided advice, expertise and moral support for this project. In particular, we must mention Raymond Vezeau, Norman Birmingham, Richard Legault, André Fouquet, Manon Harwood and Chantale Côté. Thanks to Jehangir Appoo, Jay Leopkey and Brigid Payne for technical assistance. Thanks to the personnel at the Groupe d'Intervention du Plan d'Action Saint Laurent and the Ministère de l'Environnement of the province of Québec for providing us with essential internal documents. This project was funded by a St. Lawrence Centre - Natural Sciences and Engineering Research Council of Canada research partnership grant to JBR and a Fondation Canadienne d'Aide à la Recherche grant to PAW.

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CHAPTER 4

SORPTION OF ORGANIC GENOTOXINS TO PARTICULATE MATTER IN INDUSTRIAL EFFLUENTS

INTRODUCTORY REMARKS

The material presented in this chapter was originally included in a single manuscript that contained all the industrial effluent results (chapters 3 and 4). Reviewers of the manuscript suggested dividing the material into two complementary manuscripts. This chapter contains all the material that concerns the partitioning behaviour of genotoxins in industrial effluents.

As the extension and complement to material presented in chapter 3, this chapter is also *in press* in volume 26 of *Environmental and Molecular Mutagenesis*. Again, Prof. Joseph Rasmussen and Dr. Christian Blaise played a role in the development of the ideas presented. Again, they are second and third co-authors.

Appendix 6 contains the data used to generate the models, figure and tables.

ABSTRACT

In an earlier work (White et al., 1995) we examined the genotoxicity of dichloromethane extracts from a variety of industrial effluent samples. In this companion work, we used the SOS Chromotest to investigate the sorption of the extracted genotoxins to effluent suspended particulate matter. The affinity of the genotoxins for particulate matter is expressed as a genotoxicity sorption partition coefficient ($K_{d\text{-genotox}}$). The results indicate that industries known for their emission of combustion by-products such as polycyclic aromatic hydrocarbons often have high $K_{d\text{-genotox}}$ values ($\geq 10^6$). These include metal refining and founding industries as well as metal surface treatment facilities. In contrast, $K_{d\text{-genotox}}$ values for pulp and paper mills and sewage treatment facilities are several orders of magnitude lower ($\leq 10^4$). In several cases the calculated $K_{d\text{-genotox}}$ values are in agreement with the K_{ow} 's of genotoxic substances isolated from genotoxic industrial waste samples studied by other researchers. The sorption partition coefficient, in conjunction with the concentration of available particulate matter, was used to determine the percent of organic genotoxins adsorbed to effluent suspended particulate matter. Values range from 2.3% to 99.8%. High values ($>70\%$) were obtained for metal surface treatment, inorganic and organic chemical production facilities. Low values ($<30\%$) were obtained for sewage treatment facilities and pulp and paper mills. The results also demonstrate the effect of variations in the concentration of available particulate matter on the genotoxicity of both aqueous and particulate extracts. The results suggest that the sorptive properties of the particulate matter itself is reduced when the concentration of particulate matter is very high (>1000 mg per L). The use of sorption partition information in inferring the physical-chemical nature of the putative genotoxins, and the implications of the results for assessing the hazard posed to aquatic biota by industrial genotoxins are discussed.

INTRODUCTION

Industrial wastewaters often contain high concentrations of suspended particulate matter (up to 10^3 mg per L) characterized by a high surface area to volume ratio. A recent Environment Canada survey indicated that 35 industries of concern discharge a total of 240 metric tons of particulate matter per day into the St. Lawrence river (GIPASL, 1993). Very efficient chemical processes occurring at the interface between the solvent (water) and the surface of these suspended particles regulate the partitioning of contaminants between the dissolved and particle-associated phases (Stumm, 1992). Karickhoff et al. (1979) expressed the magnitude of the association between a solute and a suspended particulate adsorbent as the sorption partition coefficient (K_d or K_p), the slope of a linear sorption isotherm. The magnitude of the K_d value is determined to a large extent by a single property of the solute, its octanol-water partition coefficient (K_{ow} or P_{oc}) (Briggs, 1973; Chiou et al., 1977; Karickhoff et al., 1979; Schwarzenbach and Westall, 1981; Allan, 1986; Bourg, 1989). To a lesser extent, the K_d value is determined by the properties of the adsorbent, including its organic carbon content (Lambert et al., 1965; Lambert, 1968; Karickhoff et al., 1979; Karickhoff, 1981; Schwarzenbach and Westall, 1981), its particle size and chemical composition (Richardson and Epstein, 1971; Choi and Chen, 1976; O'Connor and Connolly, 1980), as well as the concentration of available adsorbent (Cox, 1970; O'Connor and Connolly, 1980).

The equilibrium distribution of solutes between the dissolved and particle-associated phase of industrial effluents is of profound toxicological importance. The degree of solute-particle association can regulate the residual concentration, physical movement, biological availability and ultimate fate of the solute in the receiving water (Stumm, 1992; Allan, 1986; Bourg, 1989). Substances with high sorption partition coefficients are only sparingly soluble in water. As such, they frequently pose a greater hazard to biota that are in intimate contact with sedimented particulate matter (McLeese et al., 1980; Oliver, 1984; Allan, 1986; Tessier and Campbell, 1987; Ciborowski and Corkum, 1988). At the present time, our knowledge about the sorptive characteristics of genotoxicants in industrial wastes and effluents is extremely limited. Some genotoxic and carcinogenic organic compounds that have been isolated from industrial wastewaters (e.g. polycyclic and heterocyclic aromatic compounds, etc) have very low water solubilities and consequently high sorption partition coefficients (Mackay et al., 1992). In contrast, several researchers have shown that the mutagenic constituents of pulp and paper mill effluents are surprisingly water soluble (Kringstad et al., 1984; Møller et al., 1986) and would likely have low sorption partition coefficients. With the exception of Metcalfe et al. (1985), few researchers have ever

attempted to use a short-term genotoxicity assay to investigate the genotoxic activity of the suspended particulate component of industrial wastewaters *per se*. Although Metcalfe et al. (1985) investigated the mutagenicity of particulate material in several petroleum refinery effluents, they did not compare the concentrations of particle-associated and dissolved mutagens. White et al. (1995) investigated the genotoxicity of both dissolved and particle-associated organic compounds extracted from a wide range of industrial wastewaters. The results obtained indicate that the genotoxins in some industrial effluent samples (e.g. Aluminum refinery samples) are predominantly associated with suspended particulate matter. In this companion work, we continue to investigate the sorptive properties of organic genotoxins present in the same industrial effluents.

MATERIALS AND METHODS

Thirty-six liters of final effluent (24-hour integrated samples) were collected from 42 industries that discharge waste into the St. Lawrence, Saguenay and Fraser rivers. A total of 50 effluent samples were collected. Descriptions of the industries are provided in White et al. (1995). The industries investigated include pulp and paper mills, petroleum refining facilities, organic chemical production facilities, inorganic chemical production facilities, metal (Al, Cu, Ag, Au, Pt, Zn, Cd, Fe and Ti) refining and founding facilities, metal surface finishing operations and municipal wastewater treatment facilities. Organic constituents of both the aqueous filtrate and the suspended particulate matter were obtained via direct partitioning into pesticide grade dichloromethane. Suspended particulates were isolated by positive pressure filtration on a glass fibre filter (Gelman type AE) with a nominal retention of 1 μ m. Aqueous filtrates were extracted under both acidic (pH = 2) and alkaline (pH = 12) conditions. All extracts were dissolved in dimethyl sulfoxide and assayed for genotoxicity using the SOS Chromotest (Quillardet et al., 1982; Quillardet et al., 1985).

Significant positive responses on the SOS Chromotest were defined as those cases where the response (SOS Induction Factor) to a given sample exceeded the upper 95% confidence limit of the solvent control at a minimum of three doses. Genotoxicity of any given sample is expressed as SRIP- the SOS Response Inducing Potency and GTOX- a measure of the degree of contamination of a given sample with SOS genotoxins. The SRIP is the initial slope of the concentration response curve of SOS Induction Factor versus sample concentration (in equivalent mg of sample). The GTOX value is the inverse of the minimal detectable genotoxic concentration (MDGC), a value described by Langevin et al. (1992). The MDGC is the concentration of sample, inferred from the fitted concentration-response curve, that can produce a response equal to the upper 95% confidence limit of the control. The units of the GTOX value are therefore equivalent mg of sample⁻¹.

Concentrations of fluorescent aromatic substances in each extract were approximated using fluorescence spectrophotometry (excitation at 380nm, emission at 430nm). Benzo(a)pyrene was used as a fluorescence standard and the results are expressed as the concentration of benzo(a)pyrene (ppb or μ g per L in dichloromethane) required to equal the fluorescence of the tested extract.

Detailed descriptions of the methods employed for effluent processing and extraction, the genotoxicity assessment (SOS Chromotest) protocol, the fluorescence spectrophotometry procedure and limitations, as well as the data analysis methods can be found in White et al. (1995).

RESULTS

The results obtained in our previous study of 50 industrial effluents revealed wide variations in genotoxic potency. Variations due to industry type, metabolic (S9) activation and extract fluorescence were thoroughly discussed in White et al. (1995) and will not be further described or discussed here. While White et al. indicated that the mean genotoxic potency of the particulate matter extracts is almost four orders of magnitude greater than that of the corresponding aqueous extracts, they did not provide a detailed investigation of the sorptive properties of industrial genotoxins.

The results presented in Figures 1 and 2 reveal that the concentration of suspended particulate matter plays a role in determining the genotoxicity of both suspended particulate extracts and aqueous filtrate extracts. The concentration of suspended particulate matter has a significant, negative effect on the genotoxic potency of the particulate matter extracts. This suggests that the pool of adsorbable genotoxins is being diluted by an excess of particulate matter. The following ANCOVA model (model (1)) relates the SRIP of suspended particulate extracts to the total suspended particulate concentration and the metabolic activation status ($r^2 = 0.53$, $n = 88$, F Ratio = 25.7, $p < 0.0001$):

$$\text{Log SRIP} = 0.86 - 1.03 * \text{TSP} + 0.43 \quad (1)$$

(If S9 Not Present)

where TSP is total suspended particulates in dry mg per L of effluent. This result is illustrated on Figure 1.

The potency of the aqueous filtrate extracts is also related to the concentration of available particulate matter. However, in this case the concentration of suspended particulate matter has a significant positive effect on the genotoxicity of aqueous filtrate extracts. This suggests that when the concentration of particulate matter is very high, sorption of genotoxins to particulate matter is reduced. The following ANCOVA model (model (2)) demonstrates the effect of both suspended particulate concentration and metabolic activation status on the SRIP of aqueous filtrate extracts ($r^2 = 0.39$, $n = 55$, F Ratio = 16.7, $p < 0.0001$):

$$\text{Log SRIP} = -5.42 + 0.42 * \text{TSP} + 0.86 \quad (2)$$

(If S9 Not Present)

This result is illustrated on Figure 2.

For a given adsorbent, the sorption partition coefficient (K_d) can be calculated as the ratio of the equilibrium concentration of solute associated with the adsorbent, to the

Figure 1. The effect of suspended particulate concentration and S9 presence on the potency of suspended particulate matter extracts. The fitted lines represent the ANCOVA model (model (1)) described in the text. The difference in potency due to S9 presence is represented by the difference in intercept. Dotted line- with S9 activation; Solid line- without S9 activation. The following abbreviations are used: Sew- sewage treatment facilities, PP- pulp and paper plants, Al- aluminum refining plants, M- other metal refining plants, Pe- petroleum refining facilities, Surf- surface treatment facilities, In- inorganic chemical production facilities, Org- organic chemical production facilities.

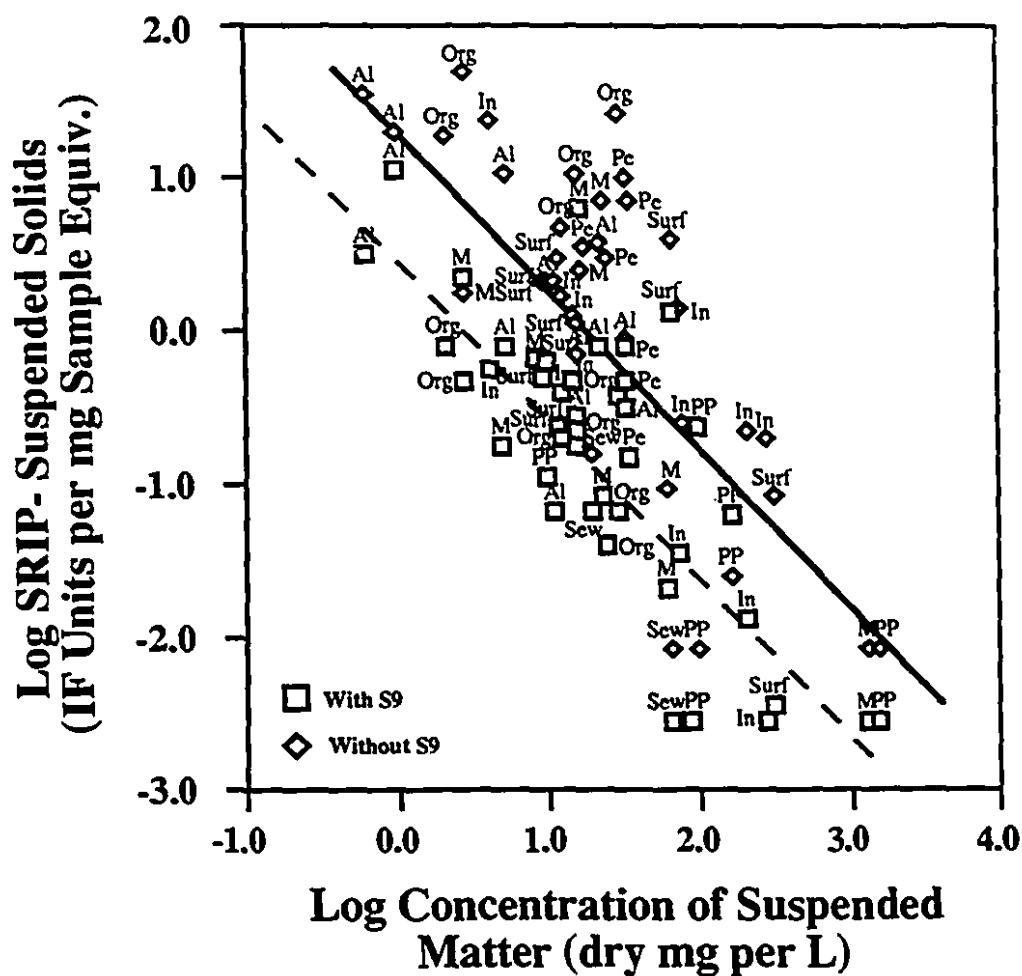
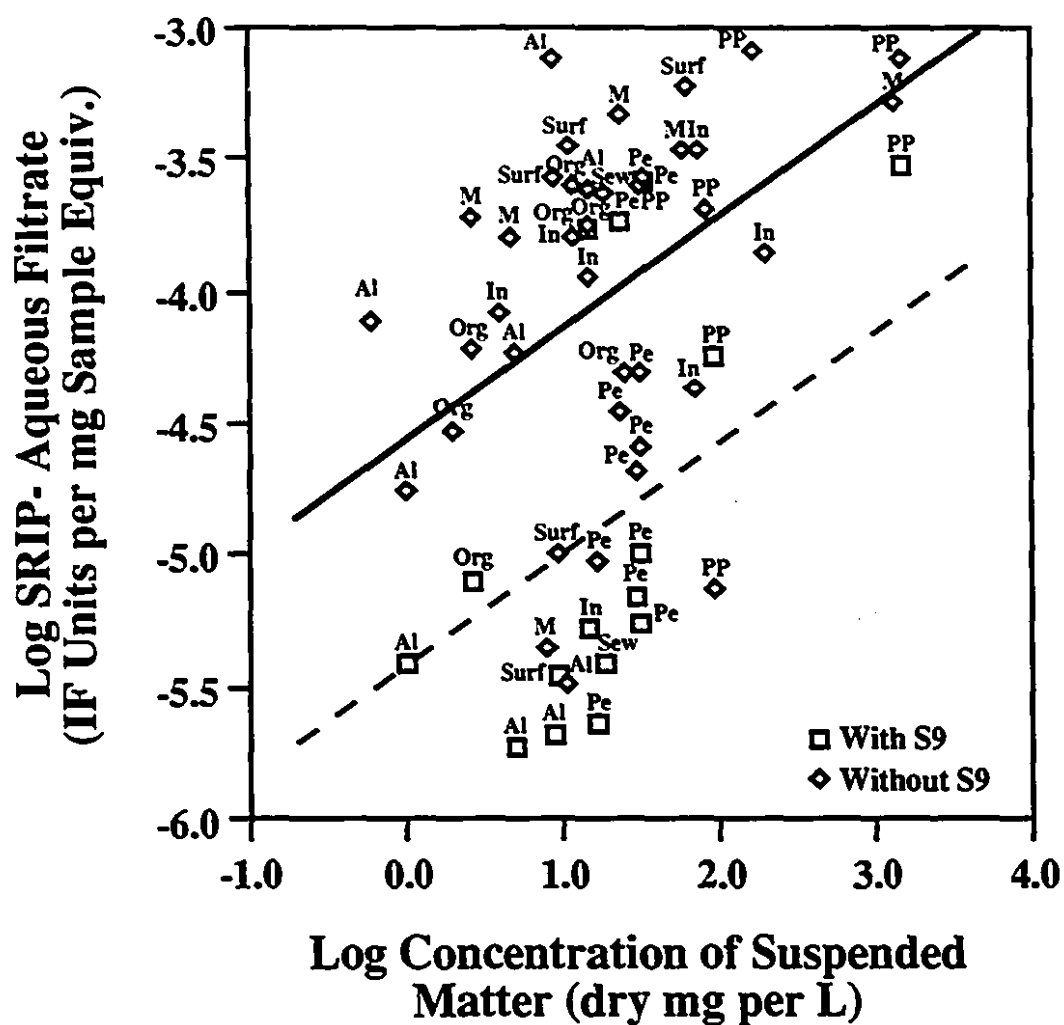


Figure 2. The effect of suspended particulate matter concentration and S9 presence on the potency of aqueous filtrate extracts. The genotoxic potency values included represent the most potent aqueous fraction (i.e. acid or alkaline) associated with each effluent sample. The fitted lines represent the ANCOVA model (model (2)) described in the text. The difference in potency due to S9 presence is represented by the difference in intercept. Dotted line- with S9 activation; Solid line- without S9 activation. Abbreviations used are the same as in Figure 1.



equilibrium concentration of solute in solution (Karickhoff et al., 1979). In the context of this study, the magnitude of the difference in GTOX between aqueous extracts and suspended particulate extracts can be expressed as a genotoxicity sorption partition coefficient- $K_{d\text{-genotox}}$. $K_{d\text{-genotox}}$ values were calculated according to equation (3).

$$K_{d\text{-genotox}} = \frac{\text{GTOX}_{\text{suspended particulates}}}{\text{GTOX}_{\text{aqueous samples}}} \quad (3)$$

When both aqueous extracts (i.e. acid and alkaline extracts) from a given sample tested positive, the denominator was a mean of the two GTOX values obtained. $K_{d\text{-genotox}}$ values ranged from a low of 78 for Pulp and Paper Industry #6 to a high of 1.5×10^7 for Organic Chemical Facility #6.

In a similar manner, the magnitude of the difference between the fluorescence of the aqueous extracts and suspended particulate extracts can be expressed as a sorption partition coefficient. $K_{d\text{-Fluor}}$ values, which quantify the partitioning of fluorescent substances in the effluent samples, were calculated according to equation (4).

$$K_{d\text{-Fluor}} = \frac{\text{Fluorescence of suspended particulate extracts}}{\text{Fluorescence of aqueous extracts}} \quad (4)$$

$K_{d\text{-Fluor}}$ values ranged from a low of 470 for surface treatment industry #4 to a high of 1.16×10^7 for Aluminum industry #2.

While the equilibrium partitioning of genotoxins and fluorescent aromatic substances are reflected by $K_{d\text{-genotox}}$ and $K_{d\text{-Arom}}$, these calculated partition coefficients do not indicate the fraction of genotoxins or fluorescent aromatics that are adsorbed to suspended particulates. If equilibrium is always obtained between the particulate and aqueous phases, then the amount of a substance associated with the adsorbent can be calculated from the sorption partition coefficient and the concentration of available particulates (Allan, 1986). For example, organic chemical production facility #3 has a $K_{d\text{-genotox}}$ value of $10^{6.02}$ and 2.0 mg per L of suspended particulate matter. Therefore, for every ppb ($\mu\text{g per L}$) of genotoxin in solution, one would expect $\{10^{6.02} \text{ ppb } (\mu\text{g per kg}) \times 10^{-6} \text{ kg per mg} \times 2.0 \text{ mg}\} = 2.09 \mu\text{g}$ of the solute to be associated with the suspended particulate matter in 1 liter of effluent. Therefore, in each liter of effluent, 2.09 out of every 3.09 μg of solute (i.e. 67.8%) will be associated with the suspended particulate matter. This calculation was performed to determine the proportion of genotoxins and fluorescent substances that are adsorbed to the suspended particulate matter of each effluent. Calculated values for genotoxins ranged from 2.3% for Metal Refining Facility #3 (sample 1) to 99.8% for

Organic Chemical Production Facility #6 (sample 1). For fluorescent substances, the calculated values range from 0.7% for Surface Finishing Industry #4 to 99.4% for Aluminum Refining Facility #2 (sample 1). Table 1 summarizes the mean percentage of both genotoxins and fluorescent substances adsorbed to suspended particulates for each industry category examined. For surface treatment, inorganic chemical and organic chemical industries, the mean fraction of genotoxins adsorbed to particulates is greater than the corresponding fraction of fluorescent substances adsorbed to particulates. In the case of the metal surface finishing industries the difference in percentage is greater than 60%. Three industry types (metal refining, petroleum refining and pulp & paper) demonstrate the opposite trend. Effluents from these industries contain genotoxins that have a lower tendency to be particle-associated than the average fluorescent substance. Genotoxins in aluminum refining and sewage treatment effluents exhibit a tendency to associate with suspended particulate matter that is similar to the average fluorescent substance in their respective effluents.

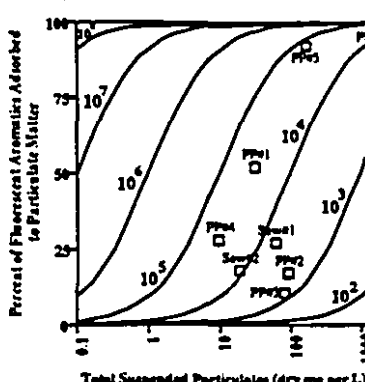
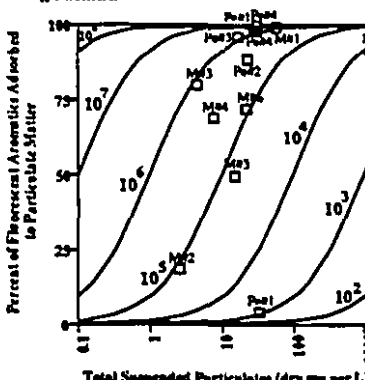
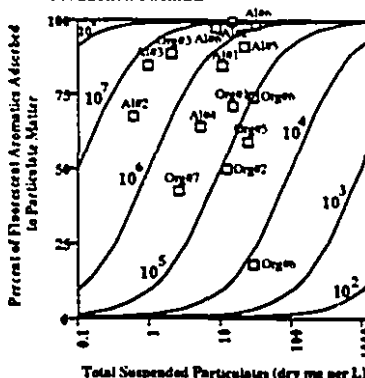
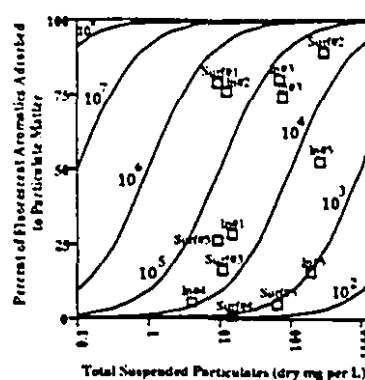
Since sorption to particulate matter is determined primarily by the K_{ow} of the solute, it is tempting to use the K_d values calculated using equations 3 and 4 to infer the physical-chemical nature of the putative genotoxins and fluorescent substances. Figure 1 indicates that increases in the concentration of suspended particulate matter can decrease the potency (in SOS Induction Factor Units per equivalent mg of sample) of suspended particulate extracts. This in turn indicates that the suspended particulate matter in the samples examined are not saturated with genotoxic material. The ability of the available suspended particulate matter to dilute the pool of adsorbable genotoxic substances complicates the interpretation of the sorption partition coefficients calculated using equations (3) and (4). If the equilibrium concentrations of particle-associated and dissolved genotoxins are determined by the amount of available particulate matter, then it is difficult to make any inferences about the physical-chemical characteristics of the putative genotoxins in any given effluent sample. Figure 3 was produced in order to permit comparisons of sorption partition coefficients while simultaneously accounting for the effect of suspended particulate concentration. The underlying curves presented in each plot represent the relationship between the fraction of any organic compound (with a sorption partition coefficient between 10^2 and 10^8) adsorbed to suspended particulates and the concentration of suspended particulates. The curves assume that the fraction adsorbed to particulates is determined solely by the sorption partition coefficient of the solute and the concentration of suspended solids. The figure permits a comparison of the sorption partition coefficients of the putative genotoxins and fluorescent substances in each sample while simultaneously accounting for the effect of the concentration of available particulate matter. The results

Table 1. Mean percentage of genotoxins and fluorescent substances adsorbed to effluent suspended particulate matter. Values were calculated using geometric mean suspended particulate matter concentrations (dry mg per L) and mean partition coefficient values. Values were rounded to the nearest whole number. Approximate concentrations of fluorescent substances were measured by fluorescence spectrophotometry. Concentration values were expressed as the concentration of benzo(a)pyrene required to equal the fluorescence of the tested extract.

Industry Type	Percent of Genotoxins Adsorbed to Suspended Particulates	Percent of Fluorescent Aromatics Adsorbed to Suspended Particulates
1) % Adsorbed Genotoxins > % Adsorbed Fluorescent Aromatics		
Surface treatment	86	23
Inorganic chemical prod.	85	43
Organic chemical prod.	72	55
2) % Adsorbed Genotoxins < % Adsorbed Fluorescent Aromatics		
Non-Al metal refining	54	77
Petroleum refining	58	92
Pulp and paper	29	50
3) % Adsorbed Genotoxins Similar to % Adsorbed Fluorescent Aromatics		
Aluminum refining	84	92
Sewage treatment	21	22

Fig. 3. The effect of suspended particulate concentration and sorption partition coefficient on the fraction of both genotoxicants and fluorescent substances adsorbed to effluent suspended particulate matter. Underlying curves illustrate the effect of sorption partition coefficient (10^2 to 10^8) and particle concentration on the percent of a substance adsorbed to particulate matter. The curves assume that the fraction of a substance carried on particulate matter is controlled solely by the sorption partition coefficient and the concentration of available particulates. Abbreviations used are the same as in Figure 1. The numbers refer to the industries listed in Table 1 of White et al. (1995).

Figure 1 is a line graph showing the relationship between the Percent of Gasoline Adsorbed to Particulate Matter (Y-axis) and Total Suspended Particulates (dry mg per L) (X-axis). The Y-axis ranges from 0 to 100 in increments of 25. The X-axis is logarithmic, ranging from 0.1 to 100. Several curves are plotted, labeled with powers of 10: 10^2 , 10^3 , 10^4 , 10^5 , 10^6 , and 10^7 . Data points are plotted for various studies, including Surtees, and for different locations like Los Angeles and San Francisco. The curves show that the percent of gasoline adsorbed increases with the total suspended particulates, and the rate of increase is higher for higher powers of 10.



indicate wide variation in both $K_d\text{-genotox}$ and $K_d\text{-Fluor}$. For example, the majority of aluminum refineries, surface treatment and inorganic chemical production industries have $K_d\text{-genotox}$ values above 10^5 . In contrast, the majority of the sewage treatment facilities and pulp & paper mills have $K_d\text{-genotox}$ values of 10^4 or less. The results show similar variability in $K_d\text{-Fluor}$ values. $K_d\text{-Fluor}$ associated with metal and petroleum refineries are high (mostly above 10^5), while surface treatment facilities, sewage treatment facilities and pulp mills are about an order of magnitude lower.

DISCUSSION

Many researchers have identified industrial genotoxins that have high sorption partition coefficients. Table 2 summarizes the octanol-water partition coefficients for several substances isolated from genotoxic industrial waste samples. With the exception of substances isolated from pulp & paper mill and munitions production effluents, most of the isolated genotoxins have K_{ow} 's well above 10^3 . Despite the presence of high K_{ow} substances and high concentrations of suspended particulate matter, few researchers have ever investigated the genotoxicity of substances adsorbed to effluent particulate matter. Metcalfe et al. (1985) demonstrated that substances adsorbed to suspended matter in three petroleum refinery effluents are weakly mutagenic in the Ames test. However, they did not attempt to compare the genotoxic contamination of the suspended matter to the aqueous supernatant of the centrifuged samples. To our knowledge only Langevin et al. (1992) ever attempted to use a short-term genotoxicity assay to compare the genotoxicity of suspended particulate matter and aqueous filtrates without any *a priori* knowledge of the genotoxic substances present. They demonstrated that in the surface waters of the St. Lawrence river, unidentified SOS genotoxins have sorption partition coefficients from 1.1×10^6 to 5.8×10^6 . In the present study, we used the same short-term genotoxicity assay as Langevin et al. to investigate the sorption of industrial genotoxins to suspended particulates in aqueous waste samples.

The results presented in Figure 1 demonstrate that the genotoxic potency of suspended matter extracts is related to the concentration of available particulate matter in the original effluent sample. As the concentration of suspended matter increases, the genotoxic potency of the particles declines. Therefore, under conditions of high particulate matter, one would expect the genotoxic contamination per unit mass of particulate matter to be low. Consequently, the calculated $K_{d-genotox}$ will also be low. This effect is apparent if the values presented in Table 1 are compared with those in Figure 3. For example, despite the fact that the average $K_{d-genotox}$ value for metal and petroleum refining industries is almost 10^5 , the low concentrations of available particulate matter results in only 50% of the genotoxins being adsorbed to particulate matter. Consequently, the genotoxic potency of the suspended matter in these industries is fairly high. Conversely, despite the fact that the concentration of particulate matter in aluminum refinery effluents is frequently lower than that found in the petroleum refinery effluents, high $K_{d-genotox}$ values (mean of 10^6) result in, on average, more than 80% of the genotoxins being adsorbed to particulate matter. Therefore, it is dangerous to use the calculated $K_{d-genotox}$ values to infer physical-chemical properties of the putative genotoxicants in the effluent samples. For example, it is tempting

Table 2. Octanol-water partition coefficients (K_{ow}) for genotoxic substances isolated from a variety of genotoxic industrial waste samples. All compounds listed are substances isolated from industrial waste samples that were simultaneously subjected to chemical characterization and genotoxicity assessment. Genotoxicity of isolated substances was confirmed using the bioassays indicated. The K_{ow} values listed can be converted to sorption partition coefficients using the empirical relationship of Karickhoff (1981). Karickhoff's empirical relationship was summarized by Allan (1986) as $K_d = 0.411 \times K_{ow} \times OC$, where OC is the fractional mass of organic carbon in the adsorbent.

Compound Identified	Log K_{ow} [†]	Sample Investigated	Genotoxicity Assay System	References
Benzo(a)pyrene	6.1	Petroleum refinery effluent Ink pigment sludge Coke plant waste	Ames ¹ test and SCE ² in CHO ³ cells	Metcalf et al. (1985) Epler et al. (1978) Houk and Claxton (1986)
Chrysene	5.9	Petroleum refinery effluent	Ames test and SCE in CHO cells	Metcalf et al. (1985) Epler et al. (1978)
Phenanthrene	4.5	Petroleum refinery effluent	Ames test and SCE in CHO cells	Metcalf et al. (1985) Epler et al. (1978)
Benz(a)anthracene	5.8	Energy production related effluents Coke plant waste		Epler et al. (1978) Houk (1992)
2-Aminoanthracene	3.3	Energy production related effluents	Ames test	Epler et al. (1978)
Acridine	3.4	Energy production related effluents	Ames test	Epler et al. (1978)
Benzo(b)fluoranthene	6.3	Coke plant waste	Ames test	Houk (1992)
Benzo(k)fluoranthene	6.6	Coke plant waste	Ames test	Houk (1992)

Table 2 continued.....

Fluoranthene	5.2	Wood preservation waste Ink pigment sludge	Ames test	Donnelly et al. (1987) Houk and Claxton (1986)
1-nitropyrene	4.3	Oil-water separator runoff (gasoline station)	Ames test	Manabe et al. (1984)
Dinitrotoluenes (various isomers)	2.0	Munitions production effluent	Ames test	Spanggord et al. (1982) Sundvall et al. (1984)
Trinitrotoluenes (various isomers)	1.7	Munitions production effluent	Ames test	Spanggord et al. (1982)
3,5-dinitrobenzoic acid	1.7	Nitrobenzoic acid and nitrotoluene production effluent	Ames test	Sundvall et al. (1984)
4,6-dinitro- <i>o</i> -cresol	2.1	Nitrobenzoic acid and nitrotoluene production effluent	Ames test	Sundvall et al. (1984)
Complex mixture ⁴	up to 3.2	Pulp and paper mill effluent	Ames test	Kringstad et al. (1984)
1,2-dichloroethane	1.5	Pulp and paper mill effluent	Ames test	Douglas et al. (1983)
2-chloropropenal	0.7	Pulp and paper mill effluent	Ames test	Kringstad et al. (1981)
1,1,1-trichloroethane	2.5	Pulp and paper mill effluent	Ames test	Douglas et al. (1983)
1,1,3,3- tetrachloroacetone	2.1	Pulp and paper mill effluent	Ames test	Douglas et al. (1983) Holmbom et al. (1984) Kringstad et al. (1981)
Neoabietic acid	6.5	Pulp and paper mill effluent	Ames test <i>Saccharomyces sp.</i> ⁵	Nestmann et al. (1979) Nestmann and Lee (1983)
Dichloro- <i>p</i> -cymene	5.4	Pulp and paper mill effluent	Ames test	Bjørseth et al. (1979)

Bromo-<i>p</i>-cymene	4.9	Pulp and paper mill effluent	Ames test	Bjørseth et al. (1979)

Bromo-<i>p</i>-cymene	4.9	Pulp and paper mill effluent	Ames test	Bjørseth et al. (1979)

⁵reversion of *trp* mutants (*S. cerevisiae* strain XV185-14C).

to infer that the genotoxins present in petroleum refinery effluents have K_{ow} 's in the 10^5 range, which in turn would reflect low water solubility (Chiou et al. 1977; Leo, 1993). However, if more suspended particulates were available then the genotoxic contamination of the average mg of particulate matter might decline. The result would be a drop in the calculated $K_{d-genotox}$ value.

Figure 3 indicates that genotoxicity sorption partition coefficients ($K_{d-genotox}$) varied from slightly above 10^2 to almost 10^7 . Superimposition of the industrial effluent results on the underlying conceptual framework provided in each plot permits $K_{d-genotox}$ values to be interpreted while simultaneously accounting for the effect of suspended matter availability. For example, the figure indicates that pulp and paper mill effluents (except PP#5) contain genotoxins with sorption partition coefficient values in the 10^4 range. Partition coefficients in this range are roughly equivalent to fairly water-soluble, low molecular weight aromatic substances such as naphthalene and biphenyl (Mackay et al., 1992). Several researchers have chemically characterized the mutagenic constituents of pulp mill effluents and demonstrated that they are primarily low molecular weight compounds that are surprisingly hydrophilic in nature (Kringstad et al., 1984; Møller et al., 1986; Kringstad and Lindström, 1984). The hydrophilic nature of genotoxins isolated from pulp and paper mill effluents is also reflected by the low K_{ow} values presented in Table 2. However, Table 2 also indicates that high K_{ow} (10^5 range) genotoxins have been isolated from pulp mill effluents. Recent chemical characterization of several pulp mill effluents support this range in K_{ow} . In a survey of 15 pulp mill effluents, Environment Canada detected a variety of known genotoxins with a wide range of sorptive properties (GIPASL, 1993). At the other extreme, $K_{d-genotox}$ values for aluminum refinery effluents almost reach 10^7 . Values of this magnitude would correspond to low solubility, high molecular weight polycyclic aromatic substances such as dibenz(*a,h*)anthracene and benzo(*ghi*)perylene (Mackay et al., 1992). Although none of the substances summarized in Table 2 were isolated from aluminum refinery effluents, genotoxins isolated from coke plant wastes would likely be similar to those produced during the electrolytic reduction of aluminum. The combustion of coal tar and pitch during aluminum refining is known to produce genotoxic, high molecular weight polycyclic aromatic substances (IARC, 1984). Figure 3 indicates that other industries which employ high temperature combustion in their production process (e.g. metal refining industries #2, #3 and #6) exhibit similar K_d values. Recent analyses of several metal refining (including Al, Cu, Fe, Zn and Ti) and founding industries in Québec have detected several high K_{ow} by-products of high temperature combustion including benz(*b*)fluoranthene, benzo(*a*)pyrene, indeno(123-*cd*)pyrene and benzo(*ghi*)perylene (GIPASL, 1993). It is interesting to note that $K_{d-genotox}$ values for petroleum refineries

presented in Figure 3 vary between 10^4 and 10^6 . Values in this range correspond to the K_{ow} values of genotoxic substances isolated from petroleum refinery and energy production related industries summarized in Table 2. Recent chemical characterization of 6 petroleum refinery effluents conducted by Environment Canada detected known genotoxins with similar sorptive properties (GIPASL, 1993).

In several instances the $K_d\text{-genotox}$ values indicated on Figure 3 do not agree with the descriptions of genotoxins found in the literature. For example, Spanggord et al. (1982) and Sundvall et al. (1984) indicate that low K_{ow} substances such as dinitrotoluenes, trinitrotoluenes, nitrobenzoic acids and nitro-cresols are responsible for a substantial amount of the genotoxic activity found in effluents of munitions production facilities. However, our results indicate that the genotoxic substances in organic chemical production facility #7 (TNT, nitroglycerin and trimethylenetrinitramine production facility) are not very water soluble and have K_{ow} values in the 10^5 range. It is interesting to note that organic chemical production facility #7 does emit some of the genotoxic substances described by Spanggord et al. (1982) (e.g. 272 grams of 2,4-dinitrotoluene per day (GIPASL, 1993)). However, at the present time there is no evidence that any of the mutagenic nitrotoluenes identified by Spanggord et al. (1982) are capable of inducing the SOS response in *E. coli* (Quillardet and Hofnung, 1993).

The data summarized in Table 2 does not include substances isolated from municipal wastewater facilities, metal surface treatment facilities or inorganic chemical production facilities. In several instances, efforts to chemically characterize effluents from these industries have identified priority contaminants (DOE, 1989) that have similar sorptive properties to the unknown genotoxins and fluorescent substances extracted in this study. For example, Pham (1993) has determined that the majority of polycyclic aromatics found in the municipal wastewater of the city of Montréal (primary treatment only) are 2-, 3- and 4-ring substances that would have K_d values in the 10^3 to 10^6 range (Mackay et al., 1992). In some instances the sorptive properties of the unidentified genotoxins and fluorescent aromatics examined in this study are distinctly different from the priority contaminants identified during routine chemical analyses. For example, despite the high K_d values suggested in Figure 3, the priority organics identified in several of the industries studied (e.g. Organic #1, Surface #1, Inorganic #2, Surface #3, Inorganic #4, Organic #5) are predominantly volatile compounds such as chloroform, acetone, trichloroethane and formaldehyde and highly water soluble, semi-volatile substances such as phenol (GIPASL, 1993). This suggests that, despite their potential hazard, the unidentified genotoxins are not listed as priorities for concern and control in Canada.

In several instances the $K_d\text{-Fluor}$ of an effluent sample was several orders of magnitude

higher or lower than the corresponding K_d -genotox value. For example, 3 of the 4 surface treatment effluents that yielded genotoxic extracts (#3, #4 and #5) have K_d -genotox values approximately two orders of magnitude higher than the corresponding K_d -Fluor. In addition, while 86% of the genotoxins in the average surface treatment effluent are adsorbed to particulate matter only 23% of the fluorescence is adsorbed. This indicates that the putative genotoxins are far less soluble than the average fluorescent substance in the effluent. This may not be surprising since surface treatment industries commonly use large amounts of low molecular weight, non-mutagenic solvents such as chlorinated benzenes and xylenes (Environment Canada, 1987). Indeed recent chemical characterizations of effluent samples from several surface treatment facilities revealed high concentrations of several chlorinated benzenes (GIPASL, 1993). Similar results were obtained for inorganic chemical production facilities #1, #2 and #4. While 85% of the genotoxins in the average effluent sample are adsorbed to particulate matter, only 43% of the fluorescence is adsorbed. Several pulp & paper mill (#1, #4 and #5), petroleum refining (#1, #2 and #4), metal refining (#1, #4 and #5) and organic chemical production (#3 and #7) effluents demonstrate the opposite trend. The putative genotoxins in these samples seem to be far more soluble than the average fluorescent substance present in the effluent. For example, while 92% of the fluorescence in the average petroleum refinery effluent is adsorbed to particulate matter, only 58% of the genotoxins are adsorbed. This may be due to a high concentration of high molecular weight, non-genotoxic aromatic compounds. Petroleum refinery effluents often contain a wide variety of high molecular weight aromatics and aliphatics. Their exact nature depends on the refining process and the boiling range of the primary products (IARC, 1989). Pulp & paper mills are known to produce a wide variety of high molecular weight compounds that originate from the decomposition of lignin.

Despite being able to account for the effect of particulate matter availability, Figure 3 does not take into account factors that can effect the sorptive properties of the adsorbent itself. Several researchers have discussed the relationship between K_d and the organic matter content of both sediment (Karickhoff et al., 1979; Karickhoff, 1981; Swarzenbach and Westall, 1981; Choi and Chen, 1976) and soils (Lambert et al., 1965; Lambert, 1968; Champion and Olsen, 1971). In addition, several researchers have discussed the effects of particle size and type on K_d (Lotse et al., 1968; Choi and Chen, 1976; Champion and Olsen, 1971; Richardson and Epstein, 1971; Karickhoff et al., 1979). Increased adsorption of pesticides with increasing clay content has been documented for both soil (e.g. Poinke and Chesters, 1973; Champion and Olsen, 1971) and sediments (e.g. Choi and Chen, 1976; Karickhoff et al., 1979). Karickhoff (1981) and Swarzenbach and Westall (1981) suggest that K_d values can be corrected for variation in sorbent organic matter content

(yielding K_{oc} where $K_{oc} = K_d \times \% \text{ organic C by weight}$). We have made no attempt to account for variations in K_d values due to changes in the organic matter content of the particulate matter. However, it should be noted that the effect of changes in particle composition and organic matter content on K_d is small (3-4 fold changes) compared to that caused by changes in the K_{ow} of the solute (orders of magnitude).

O'Connor and Connolly (1980) demonstrated that at very high particle density, K_d values tend to decrease due to an alteration in the sorptive properties of the particulate matter. This decrease likely occurs when particle-particle interactions exceed particle-contaminant interactions. An increase in particle-particle interactions results in particle aggregation and a decrease in the total surface area available for adsorption. Figure 3 indicates that at low particle density, partition coefficients are often reasonably constant (e.g. pulp & paper facilities #1, #2, #3 and #4). However, in some instances, a very high concentration of suspended particulate matter is combined with a $K_d\text{-genotox}$ value that is lower than those obtained for other, similar industries (e.g. PP #6). This may indicate a situation where the sorptive properties of the particulate matter is decreased at high particle concentrations. This type of effect is suggested by the results presented in Figure 2. The figure indicates that high potency aqueous filtrate samples were obtained from effluents with very high concentrations of suspended particulate matter. For example, the two industries with over 1000 mg per L particulate matter (Metal #5, Paper #6) yielded the highest potency aqueous filtrate extracts. In such cases, inferences about the K_{ow} of the putative genotoxins may be confounded by alteration of the sorptive properties of the adsorbent at high particle density. Further investigations would be required to determine whether the partitioning behaviour of genotoxins from samples with high suspended particulate matter are controlled by the physical-chemical properties of the solute or the adsorbent.

Langmuir (1917), in his examination of thin films on water, was one of the early researchers to describe the behaviour of hydrophobic substances in aqueous systems. More recently Tanford (1980) indicated that the insolubility of non-polar, organic solutes in aqueous systems stems from the substances' inability to disrupt the attractive forces between adjacent water molecules. The consequence is expulsion from the water and sorption to the surface of any available suspended particle. We have demonstrated that genotoxic substances extracted from a variety of industrial effluents are hydrophobic, and demonstrate a high affinity for suspended particulate matter. In several (18) effluent samples, greater than 75% of the genotoxins are associated with suspended particulates. The magnitude of this adsorption will likely have an effect on the post-emission bioavailability and hazard of industrial genotoxins. Genotoxins strongly adhered to

particulate matter will ultimately be incorporated into the bottom sediments of the river, lake or harbor into which they are discharged. As such, the substances may constitute a greater hazard to benthic rather than pelagic biota. Many researchers have described the ability of high K_{ow} substances to accumulate in both sediments (Reynoldson, 1987; Frank et al., 1979; Huggert and Bender, 1980; Kinae et al., 1981; Jungclaus et al., 1978) and benthic biota (Ciborowski and Corkum, 1988; Oliver, 1984; McLeese et al., 1980; Fowler et al., 1978). In addition, genotoxic, teratogenic and carcinogenic effects have been detected in organisms that are in intimate contact with sedimented particulate matter (Dixon, 1982; Rodriguez-Ariza et al., 1992; Warwick, 1990; Landahl et al., 1990; Metcalfe et al., 1990; Malins et al., 1984). Further research is required to determine whether biota, either benthic or pelagic, which inhabit areas located downstream from the industries examined show any signs of exposure to genotoxins.

ACKNOWLEDGMENTS

We gratefully acknowledge comments and criticisms provided by Chantale Côté. We also thank the personnel at the Groupe d'Intervention du Plan d'Action Saint Laurent and the Ministère de l'Environnement of the province of Québec for providing essential internal documents. This project was funded by a St. Lawrence Centre - Natural Sciences and Engineering Research Council of Canada research partnership grant to JBR and a Natural Sciences and Engineering Research Council of Canada grant to PAW.

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CHAPTER 5

INDUSTRIAL GENOTOXINS SORBED TO PARTICULATE MATTER IN THE ST. LAWRENCE, ST. MAURICE AND SAGUENAY RIVERS

INTRODUCTORY REMARKS

The next logical step in the research project was to expand the analyses to include samples collected in the downstream ecosystem. This chapter contains the results of SOS Chromotest analyses of suspended and sedimented particulate material collected from areas that receive the industrial discharges investigated in chapters 3 and 4.

The material presented was prepared for submission to *Environmental Toxicology and Chemistry*. Both Prof. Joseph Rasmussen and Dr. Christian Blaise played a substantial role in the development of the ideas presented. Again, they are second and third co-authors respectively.

Most of the data used to generate the models and figures can be found in Tables 1 through 5. Additional details can be found in Appendix 7.

ABSTRACT

Previous investigations of organic genotoxins discharged into the St. Lawrence river system indicated that a substantial fraction is adsorbed to suspended particulate matter. This study used the SOS Chromotest to investigate the presence, potency and behaviour of particle-bound genotoxins in the downstream ecosystem. The results indicate that while extracts of both suspended and sedimented particulate matter are genotoxic, suspended particulate matter samples are more potent in the absence of S9 activation, while the reverse is true for bottom sediments. In addition, the maximum level of SOS induction for suspended particulate matter extracts is usually several times that of the corresponding bottom sediment extracts. In the water column, concentration of suspended particulate matter available for adsorption has a negative effect on genotoxic potency. Bottom sediment results revealed a relationship between genotoxic potency and sediment organic matter content. A similar relationship for total PAH concentration indicates that the putative genotoxins have physical-chemical properties similar to that of the measured PAHs. Bottom sediment genotoxicity is also related to PAH contamination. Genotoxic equivalency factors were used to convert PAH values to their benzo(a)pyrene equivalence. Although PAH-derived genotoxicity values can explain 99% of the variability in bioassay derived values, the quantity of genotoxicity measured using PAH analyses can only account for a small fraction ($\approx 10\%$) of the SOS Chromotest measurements. Sites that receive discharges from foundries, aluminum refineries and petroleum refineries yielded several of the most genotoxic samples. Further analyses revealed that the genotoxicity of both suspended and sedimented particulate material is empirically related to the regional genotoxicity of industrial discharges. The lack of direct-acting substances in bottom sediments indicates that these substances are more easily degraded than genotoxins which require metabolic activation. Comparisons of suspended particulate and bottom sediment total genotoxicity suggests that reactive direct-acting substances may be converted to more stable pro-genotoxins upon deposition. Annual losses of genotoxins are likely less than 25%. Further research is required to investigate exposure of downstream biota and concomitant effects.

INTRODUCTION

The equilibrium distribution of organic compounds between the dissolved and particulate phases of surface waters is regulated by efficient chemical processes occurring at the solid-water interface (Stumm, 1992). The degree to which an organic substance will be associated with particulate matter, and thus removed from the dissolved phase, is governed by several physical-chemical properties of both the sorbent and the solute. The most important of these is the K_{ow} of the solute (Karickhoff et al., 1979), a property strongly correlated with water solubility (Chiou et al., 1977). To a lesser extent, sorption is determined by the organic matter content of the sorbent (Lambert, 1968; Herbes, 1977; Karickhoff et al., 1979; Schwarzenbach and Westall, 1981), the composition of the inorganic fraction of the sorbent (Prahl and Carpenter, 1983; Karickhoff et al., 1979) and the concentration of the sorbent itself (O'Connor and Connolly, 1980). In an ecotoxicological context, the direction and magnitude of sorption to particulate matter will determine the movement, accumulation and bioavailability of contaminants in aquatic systems (McCarthy and Black, 1988; Knezovich et al., 1987; Tessier and Campbell, 1987).

Although few researchers have ever addressed the partitioning behaviour of genotoxins *per se*, many known genotoxic substances are aromatic compounds that contain 3 or more fused rings (Heflich, 1991; LaVoie et al., 1980; Woo and Arcos, 1981). As such, they will frequently have high K_{ow} 's (Leo, 1993), low water solubilities (Chiou et al., 1977) and can be expected to associate with particulate material (Karickhoff et al., 1979). Without *a priori* knowledge of genotoxin identity, White et al. (1995a) demonstrated that a substantial fraction of the genotoxins present in industrial and municipal wastewaters ($\approx 30-90\%$) are indeed associated with particulate matter. As a result, uncharacterized genotoxins discharged into surface waters can also be expected to associate with particulate material. Although researchers have not specifically addressed the magnitude and direction of this association, several studies have investigated the presence of genotoxins in freshwater and marine sediments collected from areas that receive industrial wastes. For example, Durant et al. (1992) employed the Ames/*Salmonella* mutagenicity test to detect mutagens in organic extracts of sediments from areas that receive tannery and leather finishing wastes. West et al. (1988), Balch et al. (1995) and Marvin et al. (1993) investigated mutagens in sediments collected from areas known to receive coke production and steel founding wastes.

The mechanisms through which biota can be exposed to particle-bound genotoxins *in situ* have not been investigated. However, available information indicates that intimate

contact with genotoxic sediments can have severe consequences. Several studies have revealed empirical associations between the presence of genotoxins and mutagens in sediments and the frequency of idiopathic lesions in benthic fish and invertebrates (Gardner et al., 1987; Gardner et al., 1991; Malins et al., 1984; Mix, 1986; Landahl et al. 1990). In addition, Metcalfe et al. (1990) demonstrated that extracts from Hamilton Harbour sediments can induce hepatocellular carcinomas in rainbow trout and DNA adducts in mouse cell tissue cultures. Such studies raised awareness about the potential hazards of contaminated sediments and motivated numerous attempts to isolate and identify the putative genotoxins and carcinogens. While progress has been made (e.g. Balch et al., 1995; Marvin et al., 1993), the results of surface waters and sediment analyses are rarely used to investigate source, movement or fate. In contrast, contamination of particulate material with persistent organochlorines is frequently used to provide information about source (Latimer et al., 1990), persistence (Allan, 1986) and historical patterns of deposition (Carignan et al., 1994; Durham and Oliver, 1989). In this study we examined the genotoxicity of both suspended and sedimented particulate matter collected from several sites in the St. Lawrence river system that are known to receive genotoxic industrial discharges (White et al., 1995a; White et al. 1995b). The results obtained are used to investigate the fate of industrial genotoxins adsorbed to particulate matter. In addition, we investigate empirical relationships between the genotoxicity of sediment samples and regional genotoxic loadings (White et al., 1995b).

MATERIALS AND METHODS

Study Site and Sample Collection

Samples were collected from 21 sites along the St. Lawrence river and two of its tributaries, the St. Maurice and Saguenay rivers. The region contains several industrial centers that have been identified by provincial and federal authorities as priorities for concern and control (CSL, 1992). Individual sampling sites were selected for their proximity to industries previously identified as sources of (geno)toxic contaminants (White et al., 1995b; EIS, 1992). The location of all sampling sites is illustrated on Figure 1. Additional sampling site information is provided in Table 1. Conductivity was measured according to APHA (1989a) (Orion Research Inc., Cambridge, MA). True colour was determined by the platinum-cobalt method, following filtration through a glass fibre filter with a nominal retention of 1 μm (Environment Canada, 1974).

All samples were collected during the summers of 1991 and 1992. Tracer studies conducted by Environment Canada determined that vertical mixing of discharges occurs within the first several hundred meters. This *turbulent zone of initial mixing* is followed by less rapid horizontal mixing that continues for several kilometers (Boudreault and Cantin, 1994). Collection sites were located 1000-1500 m from the effluent outfall(s). Surface water samples (≈ 60 L) from each site were collected in solvent washed, 5 gallon (18.925 L) stainless steel containers (Spartanburg Steel Products Inc., Spartanburg, SC). Suspended particulate matter was isolated via positive pressure filtration (Millipore Corp., Bedford, MA) on 292 mm glass fibre filters (Type AE, Gelman Sciences, Ann Arbor, MI). Particulate matter samples were immediately frozen and stored at -20°C until extraction. Suspended particulate matter concentrations (dry mg L^{-1}) were determined by gravimetric analysis (APHA, 1989b).

Bottom sediments were collected using a grab type 225 cm^2 Ekman sampler (Wildco Wildlife Supply Co., Saginaw, MI). Soft sediments were available at all sites except Varennes where the high current speed (> 2 knots) prevents sediment accumulation. At this site a sediment sample was collected from a common stream that receives liquid discharges from four of the five industries present. At tidal sites (Donnacona, St. Romuald, Beauport, Beaupré, La Baie and Jonquière) bottom sediments were collected from the intertidal zone at low tide. Two, 250 mL samples of surficial (top 2 cm) sediment were collected from each site. Samples were placed in glass containers, immediately frozen, and stored at -20°C until use. Water content was determined by drying 30 mL aliquots overnight at 105°C (Wetzel and Likens, 1991). Organic matter content (% dry weight) was determined by loss on ignition at 550°C (Wetzel and Likens, 1991; Håkanson and Jansson, 1983). Sand

Figure 1. Location of sampling sites. Site numbers correspond to those in Table 1. Sixteen sites (sites 1-10 and sites 13-18) are located on the St. Lawrence river between Beauharnois and Beaufré. Two sites (sites 11 and 12) are located on the St. Maurice river near La Tuque. The remaining three sites (19-21) are located along the Saguenay river between Alma and La Baie. A control site at Lake Memphremagog is not shown (≈ 100 km southwest of Montreal).

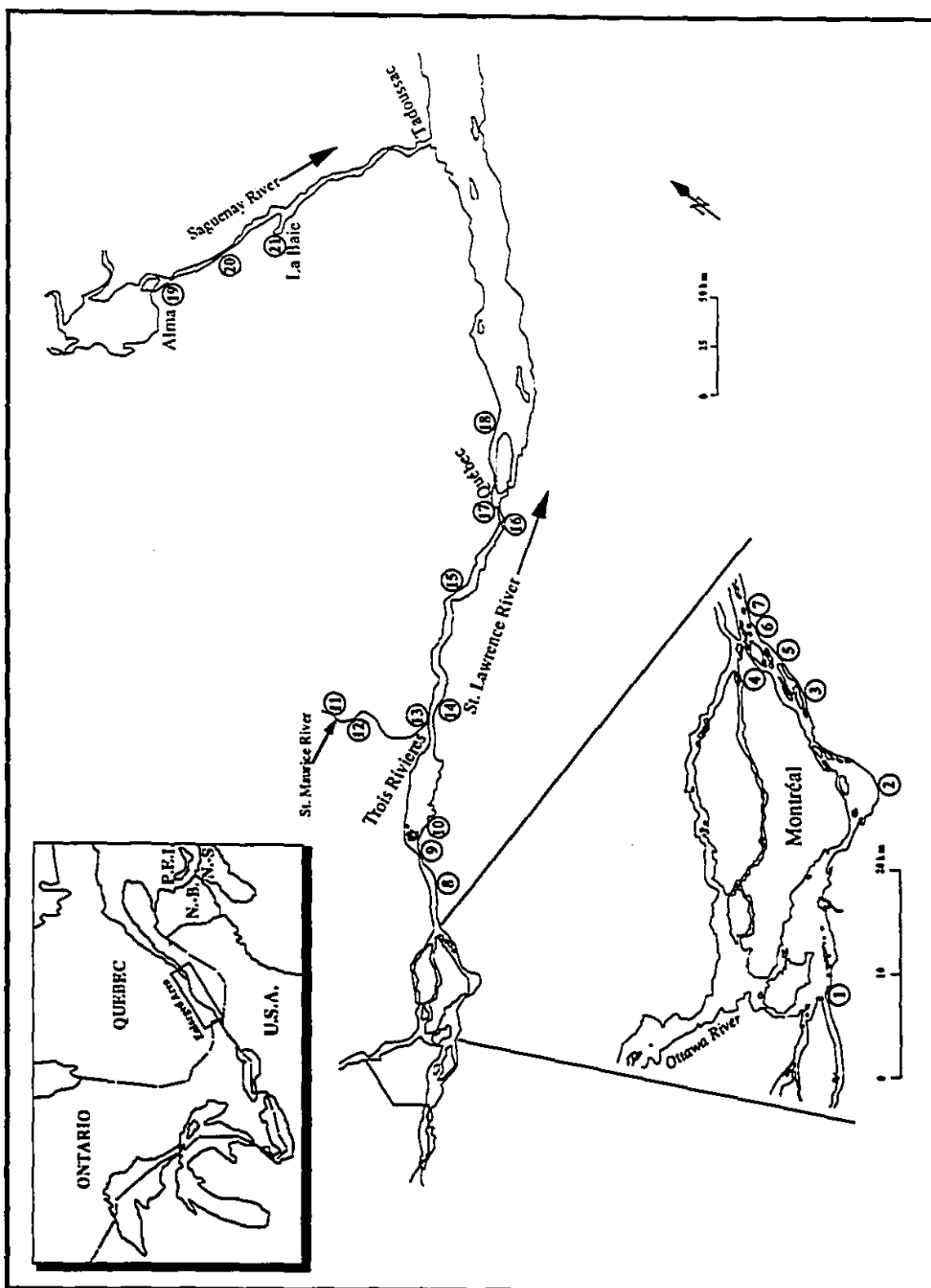


Table 1. Sampling site characteristics. Site locations are illustrated on Figure 1. Numbers adjacent to site names correspond with those on Figure 1. Conductivity and colour values are provided to indicate the water mass in which the samples were collected. Genotoxic loading values are the total, maximum loadings from dissolved acidic substances, dissolved base/neutral substances and substances adsorbed to effluent suspended solids (White et al., 1995a). Control samples (matrix blanks) of suspended particulates and bottom sediments were collected at Lake Memphremagog, a site that receives no direct industrial input.

Site	True Colour* (Pt-Co. Units)	Conductivity ($\mu\text{S cm}^{-1}$)	SPM (dry mg L ⁻¹)	Discharge† (m ³ sec ⁻¹)	Associated Industries‡	Genotoxic Loading (g BaP Equiv. day ⁻¹)
Beauharnois (1)	16.5	300	2.67	7780	PP, Al, In	372.6
La Prairie (2)	13.2	307	3.87	8478	PP, In	0.4
Longueuil (3)	14.7	300	7.85	9500	Surf, In	168.3
Montreal-East (4)	19.2	296	4.6	9548	M, Pc (2)	1234.8
Pointe-aux-Trembles (5)	16.0	311	4.29	9548	Sew	31107.0
Boucherville (6)	16.0	323	4.73	9500	Org	5.1
Varenes (7)	13.7	302	5.21	9500	Org (2), In (2), Pc	1349.5
Contrecoeur (8)	20.8	305	6.51	9700	M	2520.1
Tracy (9)	15.0	310	7.86	9705	In, M	7603.5
Sorel (10)	16.5	275	7.50	10072	M, Org	2174.9
La Tuque 1 (11) ⁰	45.4	20	52.50	496	PP	NM
La Tuque 2 (12)	47.1	28	41.00	496	PP	NM
Trois Rivières (13)	71.0	75	3.00	11500	PP, Al	4858.5
Bécancour (14)	31.5	7901	3.00	11500	Org, Al	95.7
Donnacoona (15)	41.0	85	32.50	12000	PP	254.9

Table 1 continued.....

St. Romuald (16)	33.0	210	24.70	12500	Pe	47.6
Beauport (17)	37.8	275	29.40	12600	PP	NM +
Beaupré (18)	34.8	225	177.50	12600	PP	NM +
Alma (19)	52.6	59	2.20	1685	PP, Al	36.3
Jonquière (20)	63.7	163	21.90	1700	PP (2), Al, In	4401.6
La Baie (21)	69.3	>1000 [§]	33.40	NA	PP, Al	346.1
L. Memphremagog	NM	NM	0.98	NA	None	-----

*Colour values are from NAQUADAT, 1991 and 1992.

†River discharge values are 30 year (1960-1990) summer mean values (Environment Canada, 1992).

‡Org=organic chemical production, In=inorganic chemical production, Pe=petroleum refinery, PP=pulp and paper, Sew=sewage treatment, M=metal refining and founding, Surf=metal surface treatment. Values in parentheses indicate the number of each type.

°La Tuque values are averages of 1991 and 1992 values.

¶High conductivity likely the result of discharges from a salt (NaCl) processing industry.

§Salinity at La Baie (Saguenay Fjord) = 5-10 ‰.

NA - indicates not available or applicable.

NM - indicates not measured, + indicates that genotoxic substances identified in effluent samples (EIS, 1992).

content was determined by wet sieving (brass sieves - Endecotts Ltd., London, England). Inorganic material larger than 2 mm was denoted fine gravel (Håkanson and Jansson, 1983). Inorganic material smaller than 2 mm and greater than 63 μm was denoted sand (Håkanson and Jansson, 1983). The physical-chemical characteristics of each sediment sample are summarized in Table 2.

Preparation of Sediment Extracts

Suspended particulate matter and bottom sediments were extracted using a combined blender/sonication procedure. The method, fully described in White et al. (1995b) is a variation on those of Marble and Delfino (1988), Maggard et al. (1987) and Williams (1989). Briefly, wet samples were blended with 400-500 mL of dichloromethane (CAS # 75-09-2, Anachemia Science, Montreal) in stainless steel cups using a high-speed (3500 rpm) industrial blender (Eberbach Corp., Ann Arbor, MI). Each sample was blended for 3 x 4 minutes and subsequently sonicated (ultrasonic cell disrupter- Branson Sonic Power Co., Danbury, CT) on ice for 2 x 3 minutes. Blending and sonication periods were alternated with 3-4 minute cooling periods. Solid material, including particulate matter and filter fragments, were removed via filtration through coarse sintered glass. For bottom sediments, a 2 cm layer of Kieselguhr (CAS # 61790-53-2, diatomaceous earth - A&C American Chemicals, Montreal) was used as a filtration aid. Extracts were dried with anhydrous sodium sulfate (CAS # 7757-82-6, Mallinckrodt Specialty Chemicals, Mississauga, Ontario) and reduced to approximately 5 mL by rotary evaporation at 30°C (Büchi Laboratorium-Technik AG, Flawil/Schweiz, Switzerland). All extracts were taken to dryness under vacuum at low temperature (Speedvac AS290 Concentrator, Savant Ltd., Farmingdale, NY). Prior to resuspension, the mass of the extractable residue was determined gravimetrically. Final extracts were resuspended in dichloromethane (Sigma Chemical Co., St. Louis, MO) in preparation for gel permeation clean-up.

The extraction method was validated by spiking a sample of bottom sediment (54% water, 4.4 % organic matter) from the St. Maurice river and a sample of suspended particulate matter from lake Memphremagog with a mixture of 15 polycyclic aromatic hydrocarbons (Supelco PAH Mix 4-8905, Supelco Canada Inc., Oakville, Ontario). Mean recoveries of 3-, 4- and 5-ring PAHs from the spiked bottom sediment were 66%, 84% and 86% respectively. Mean recoveries of 3-, 4- and 5-ring PAHs from the spiked suspended particulate matter were 60%, 79% and 89% respectively.

All labware were precleaned with pesticide grade acetone and hexane (Anachemia Science, Montreal). Glassware were baked at 350°C to remove solvent traces and covered with pre-cleaned aluminum foil. Glass fibre filters, sodium sulfate and Kieselguhr were

Table 2. Physical-chemical characteristics of bottom sediment samples. Granulometric measurements were only performed on the nine samples that yielded a significant genotoxic response.

Site	Water Content (%)	LOI (% Dry Wt)	Sand (% Dry Wt)	Fine Gravel (% Dry Wt)	Material > 63 µm (% Dry Wt)	Material < 63 µm (% Dry Wt.)
<u>Samples that Elicited Positive or Marginal Responses</u>						
Montreal-East	37.7	2.7	93.6	0.1	93.7	6.3
Jonquière	61.3	5.3	38.4	0.0	38.4	61.6
Beauport	27.9	0.7	98.7	0.9	99.6	0.4
Boucherville	72.6	10.1	65.3	8.4	73.7	26.3
La Baie	30.0	2.2	68.1	2.8	70.9	29.1
St. Romuald	24.8	1.4	49.5	49.5	99.0	1.0
Donnacoona	32.7	1.6	63.7	9.5	73.2	26.8
Trois Rivières	43.9	3.4	81.6	0.0	81.6	18.4
Beaupré	52.9	3.5	16.4	0.0	16.4	83.6
<u>Samples That Elicited Negative or Erratic Responses</u>						
Beauharnois	77.7	10.4				
La Prairie	74.6	8.5				
Longueuil	65.9	7.5				
Pointe-aux-Trembles	33.8	1.4				

Table 2 continued.....

Varennes	31.3	1.9
Contrecoeur	70.0	5.9
Tracy	66.4	4.0
Sorel	53.7	2.5
Bécancour	60.1	6.6
Alma	44.8	7.6
La Tuque 1-1991	53.5	4.0
La Tuque 1-1992	65.4	7.9
La Tuque 2-1991	69.5	7.0
La Tuque 2-1992	57.9	3.9
Memphremagog	81.2	17.4

combusted at 450°C before use.

Gel Permeation Chromatography

Lipids and other high molecular weight substances (e.g. carotenoids, chlorophylls) can interfere with the performance of the aqueous genotoxicity assay. These substances were removed using GPC (gel permeation chromatography) on Bio-Beads™ SX-3 (Bio-Rad Laboratories, Mississauga, Ontario). Although other methods can be employed to remove interfering substances, GPC is nondestructive and, other than molecular size, it makes no assumptions about the physical/chemical properties of the toxic substances present in the complex extract. The method employed is a variation on EPA method 3640 (USEPA, 1986) incorporating some of the modifications described by Czuczwa and Alford-Stevens (1989) and Engel (1985). Briefly, ≈50 g of Bio-Bead™ resin was permitted to swell overnight in 45% dichloromethane in hexane (Anachemia Science, Montreal) and packed into 35 cm (≈175 mL) glass columns (Corning, Richmond Hill, Ontario). Sediment extracts were applied to pre-calibrated columns and eluted under gravity at 1 mL min⁻¹. The initial fraction (80-90 mL) was collected, weighed and discarded. Sample elution continued to a total elution volume of 250 mL. Eluted samples were reduced to approximately 5 mL by rotary evaporation at 30°C and taken to dryness under vacuum at low temperature. The weight of the post-GPC residue was measured, and the remaining extract dissolved in high-purity DMSO (dimethyl sulfoxide) (Sigma Chemical Co., St. Louis, MO). For bottom sediments, an aliquot of the post-GPC residue equivalent to 5 g of dry sediment was retained for PAH (polynuclear aromatic hydrocarbon) analyses.

GPC columns were calibrated with a dichloromethane solution containing 100 mg mL⁻¹ corn oil, 2 mg mL⁻¹ pentachlorophenol (CAS # 87-86-5, Aldrich Chemical Co., Milwaukee, WI), 2 mg mL⁻¹ bis(2-ethylhexyl) phthalate (CAS # 117-81-7, Aldrich Chemical Co., Milwaukee, WI), 0.04 mg mL⁻¹ perylene (CAS # 198-55-0, Aldrich Chemical Co., Milwaukee, WI) and 0.1 mg mL⁻¹ sulfur (CAS # 7704-34-9, Anachemia Science, Montreal). Flow conditions (under gravity) and lipid load were adjusted to optimize the separation of corn oil lipids from bis(2-ethylhexyl) phthalate. Optimal results were obtained with a flow rate of 1 mL min⁻¹ and a lipid load that did not exceed 250 mg. Elution of calibration compounds was monitored by absorbance at 254 nm (Beckman DU-70 UV-VIS spectrophotometer, Beckman Instruments, Palo Alto, CA). Dump volumes were adjusted to permit >85% removal of the lipids and >85% recovery of the bis(2-ethylhexyl) phthalate (USEPA, 1986). Dump volumes ranged from 80-90 mL with complete sulfur elution at approximately 160 mL.

SOS Chromotest Analyses

Genotoxicity was measured using the SOS Chromotest (Quillardet et al., 1982; Quillardet and Hofnung, 1985). The test involves incubation of *Escherichia coli* PQ37 (*lacΔU169 uvrA rfa sulA::Mud(Ap,lac) cts* PhoC) with the pure compound or complex mixture to be investigated. The *sulA::Mud(Ap lac)* fusion places the production of functional β -galactosidase under the express control of the SOS response pathway which is induced by DNA damaging agents (Walker, 1984). Post-exposure β -galactosidase activity reflects the genotoxicity of a given sample. Alkaline phosphatase activity provides an indirect measure of cell viability (Quillardet et al., 1982). Each extract was tested both in the presence and in the absence of a post mitochondrial supernatant (S9 fraction) obtained from Arochlor 1254 induced rat liver (Molecular Toxicology Products Inc., Annapolis, MD). S9 mixture was prepared following the method of Quillardet and Hofnung (1985). Final S9 concentration was 1% v/v. 4-Nitroquinoline-1-oxide (CAS # 56-57-5, Sigma Chemical, St. Louis, MO) and 2-aminoanthracene (CAS # 613-13-8, Sigma Chemical, St. Louis, MO) were used as positive controls.

The semi-automated SOS Chromotest protocol employed is discussed in detail in White et al. (1995c). Briefly, a 16 hour culture (37°C) of *E. coli* PQ37 was diluted in fresh medium (Luria-Bertani (LB) broth + 20 μ g per mL ampicillin) to an optical density (600 nm) of 0.05-0.075. The sample (or solvent blank), dissolved in 10 μ L DMSO was added to 190 μ L of diluted culture (plus S9 if desired) present in the first well of each dilution series and serially diluted to provide a total of six, 2-fold dilutions. Maximum tested concentrations were $\approx$ 10 g dry material per assay mL for bottom sediments and $\approx$ 1 g dry material per assay mL for suspended particulates. Twelve control wells received PQ37 culture and blank solvent. Each extract was tested in duplicate at the highest possible concentration range. The final background concentration of DMSO was 5% v/v.

Microplates were incubated for 3 hours at 37°C and subsequently centrifuged at 2000 g for 20 minutes. The supernatant was removed and the bacterial pellet resuspended in 100 μ L of 200 mM (Tris[hydroxymethyl])-aminomethane (CAS # 77-86-1) pH 7.5 and 100 μ L of cell lysis/enzyme assay buffer (40% v/v methanol (CAS # 67-56-1), 0.5% v/v toluene (CAS # 108-88-3), 10% v/v N,N-dimethyl formamide (68-12-2), 4 mg/mL 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-Gal, CAS # 7240-90-6)), 1 mg/mL *p*-nitrophenyl phosphate (CAS # 4262-83-9)). Plates were mixed for 60 seconds (Titertek microplate vortex, Flow Laboratories, Lugano, Switzerland) and incubated (37°C) for 60 minutes after which *initial* optical density readings (Titertek Multiskan MCC/340 Microplate Spectrophotometer) were taken (620 nm and 405 nm). Final optical density readings were taken 120 minutes after cell resuspension and lysis. Optical density values recorded at 620

nm reflect the activity of β -galactosidase, those recorded at 405 nm measure the activity of alkaline phosphatase. Calculation of net optical density values (120 min value - 60 min value) allowed the contribution of any remaining colored substances to be accounted for prior to data analysis.

Data Analyses

Induction of the SOS response pathway at a sample concentration C, denoted $R(C)$, is the ratio of net (120 min - 60 min) β -galactosidase activity to net alkaline phosphatase activity. To account for the background induction of the *sulA* gene, a normalized SOS response induction factor was calculated as $IF = R(C)/R(0)$, where $R(0)$ is the ratio of β -galactosidase to alkaline phosphatase activity averaged over the 12 controls which received blank solvent. A normalized induction factor that exceeded the upper 95% confidence limit of the control was regarded as a significant positive. Since normalized induction factor values are ratios of two means, confidence limits were calculated according to Welsch et al. (1988). Concentration response curves of normalized induction factor (IF) against sample concentration (equivalent g or mg dry particulate matter per assay mL) were inspected and the results qualitatively categorized as follows-

- 1) Negative- induction factor never exceeded the upper confidence limit of the control;
- 2) Marginal- induction factor exceeded the upper confidence limit of the control at one or two concentrations only;
- 3) Positive- induction factor exceeded the upper confidence limit of the control at a minimum of three doses;
- 4) Erratic- induction factor exceeded the control at several concentrations, but the concentration-response relationship was erratic and could not be interpreted.

Every effort was made to remove erratic responses from the data set. All samples that produced erratic responses, especially where growth inhibition was substantial, were reanalyzed at one-tenth and/or one-hundreth the original concentration range. Genotoxic potency values were calculated for each sample that elicited a significant positive response. The SOS response inducing potency (SRIP), expressed as SOS Induction Factor units per equivalent g of dry starting material, is the initial slope of the concentration response curve. The minimum detectable genotoxic concentration (MDGC) is the concentration of extract (equivalent g dry starting material per assay mL), inferred from the fitted concentration-response curve, required to produce an induction factor that equals the upper 95%

confidence limit of the control. The maximum level of SOS induction (MaxIF) was also noted and recorded.

When concentration response curves were distinctly nonlinear, exhibiting zero-order kinetics at high sample concentration, they were analyzed by iterative, non-linear regression employing a least squares loss function (SAS Institute, 1988). In these cases, the SRIP was calculated according to Langevin et al. (1992). When the range of concentrations tested did not result in a full hyperbola, the SRIP was determined by ordinary, least squares linear regression.

All data analyses were performed using the SAS system version 6.10 for OS/2™ (SAS Institute, 1993). Analysis of covariance (ANCOVA) and least squares linear regression were used to investigate empirical patterns in the results. Where necessary the data were log transformed to equalize the variance across the range of observations and meet the assumptions of least squares regression and ANCOVA (Ricker, 1973). The residual error associated with all regression and ANCOVA models were assumed to be independent and normally distributed. Normality was assessed using the Shapiro-Wilk test and visual examination of a normal probability plot (SAS Institute, 1988; Zar, 1984).

Polycyclic Aromatic Hydrocarbon (PAH) Analyses

PAH analyses were conducted for 15 bottom sediment extracts. For each sample analyzed, an aliquot of the post-GPC material equivalent to 5 g of dry sediment was dissolved in 100 µL pesticide-grade benzene (Anachemia Science, Montreal). Samples were spiked with d₁₀-fluoranthene (Supelco Canada, Oakville, Ontario), the internal standard. PAH concentrations were measured via gas chromatography (GC)/mass spectrometry (MS) (HP 5890 Series II GC with HP 5971A MS, Hewlett-Packard Canada, Ltd., Mississauga, Ontario) in SIM (selected ion monitoring) mode. Detection limits were ≈ 0.2 ppb (ng per g dry wt.). A list of the PAHs analyzed is provided in Table 3. 1 µL of each sample was injected *on column* with the following GC/MS conditions:

Column- J&W DB-5 30 m x 0.25 mm (Alltech Associates Inc., Guelph, Ontario);

Mass Range- 127-302 amu;

Temperature program- 80°C for 2 min, 80°-230°C at 10°C per min,
230°-300°C at 20°C min⁻¹;

Carrier gas- He at 8 psi, 80°C with constant flow.

Table 3. Polycyclic aromatic hydrocarbons investigated in this study. All require S9 metabolic activation. Genotoxic equivalency factors (GEF) reflect the potency of each PAH relative to BaP. In concept, they are analogous to the toxic equivalency factors (TEF) used to calculate concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxic equivalents in fish and bird tissues (van den Heuval, 1994; Tillitt et al., 1991; Ankley et al., 1991). GEF values were used to convert PAH measurements to their BaP equivalence.

Compound	CAS #*	Molec. Wt (g/mole)	Genotoxic?/ Carcinogenic?‡	GEF¶	Detected?
Naphthalene (NAPH)	91-20-3	128.2	0/0	0.00	Yes
Acenaphthylene (ACENY)	208-96-8	152.2	NA	0.00	Yes
Acenaphthene (ACEN)	83-32-9	154.2	0/NA	0.00	Yes
Fluorene (FLU)	86-73-7	166.2	0/I	0.00	Yes
Phenanthrene (PHEN)	85-01-8	178.2	++?/I	0.101	Yes
Anthracene (ANTH)	191-26-4	178.2	+/0	0.0330	Yes
Fluoranthene (FLUOR)	206-44-0	202.3	++/0	0.398	Yes
Pyrene (PYR)	129-00-0	202.2	++?/0	0.0534	Yes
Benzo(c)phenanthrene (B(c)P)	195-19-7	228.3	+/I	1.50	Yes
Benz(a)anthracene (B(a)A)	56-55-3	228.3	++/++	0.0211	Yes
Chrysene (CHRY)	218-01-9	228.3	++?/+	0.104	Yes
Benzo(j)fluoranthene (B(bjk)F)	205-82-3	252.3	+ /++	0.262	Yes ^Q
Benzo(h)fluoranthene	205-99-2	252.3	++?/++	--	--
Benzo(k)fluoranthene	207-08-9	252.3	+ /++	--	--
Benzo(a)pyrene (B(a)P)	50-32-8	252.3	+++ /++	1.000	Yes

Table 3 continued.....

Indeno(1,2,3- <i>cd</i>)pyrene (I(123cd)P)	193-39-5	276.3	+ / ++	0.0283	Yes
Dibenz(<i>a,h</i>)anthracene (DB(ah)A)	53-70-3	278.3	++ / ++	0.0381	Yes
7,12-Dimethylbenz(<i>a</i>)anthracene (DMBA)	39834-38-3	256.3	+ / ++	0.208	No
3-Methylcholanthrene (3MC)	56-49-5	268.3	+ / ++	0.215	No
Benzo(<i>ghi</i>)perylene (B(<i>ghi</i>)P)	191-24-2	276.3	+ / I	0.0249	Yes
Dibenz(<i>a,h</i>)pyrene (DB(ah)P)	189-64-0	302.4	+ ? / ++	0.0381	No
Dibenz(<i>a,l</i>)pyrene (DB(al)P)	191-30-0	302.4	+ / ++	1.19	Yes
Dibenz(<i>a,i</i>)pyrene (DB(ai)P)	189-55-9	302.4	+ / ++	0.228	No

*Chemical Abstracts Service registry number.

†Based on information from IARC (1983); Hermann (1981); Sakai et al. (1985); LaVoie et al. (1980); Mersch-Sundermann et al. (1992); Mersch-Sundermann et al. (1993); Gold et al. (1984); Gold et al. (1986). Genotoxicity symbols are: 0 - no evidence of genotoxicity, + - indicates positive response on prokaryotic short-term tests, ++ - indicates positive response on both prokaryotic and eukaryotic short-term tests, ? - indicates conflicting results reported in the literature. Carcinogenicity symbols are: 0 - no evidence of carcinogenicity, I - inadequate evidence for evaluation, + - limited evidence of carcinogenicity in experimental animals, ++ - sufficient evidence of carcinogenicity in experimental animals. NA indicates data not available.

‡Data are from measurements made in our laboratory as well as Mersch-Sundermann et al. (1992), Mersch-Sundermann et al. (1993), Mersch-Sundermann et al. (1994), Ohta et al. (1984), Von der Hude et al. (1988) and Quillardet et al. (1985).

§Result reported as the sum of benzo(*b*)fluoranthene, benzo(*k*)fluoranthene and benzo(*j*)fluoranthene.

The Following abbreviations are used throughout the text-

SRIP = SOS Genotoxic potency in Induction Factor Units per equivalent g dry weight of starting material. MaxIF = maximum SOS induction factor observed. TSP = concentration of total suspended particulates in dry mg per L. BaP = benzo(a)pyrene. BaP Equiv. = benzo(a)pyrene equivalents. PAH = polycyclic aromatic hydrocarbons. BSED = bottom sediment. SPM = suspended particulate matter. LOI = loss on ignition at 550°C. ppb = parts per billion or ng per g dry weight. ppm = parts per million or µg per g dry weight. DMSO = dimethyl sulfoxide. DCM = dichloromethane.

RESULTS

It is not possible to examine St. Lawrence river samples that receive discharges from one isolated industry. Along its course, the river continually receives municipal and industrial discharges from a wide range of sources. In addition, water entering the St. Lawrence river system comes from the upstream Great Lakes. The Great Lakes are known to contain a wide range of industrial contaminants (Environment Canada et al., 1991). In order to examine the impact of one isolated industry on the genotoxicity of downstream SPM and BSED, we began our investigations at La Tuque, Québec. The site contains a single isolated Kraft paper mill that has been previously shown to emit substances that have adverse effects on the downstream biota (Downing, 1993; Hodson et al., 1992). Samples of both SPM and BSED were collected 500 m above (La Tuque site 1 - site 11) and 500 m below (La Tuque site 2 - site 12) the final effluent outfall. The SPM results obtained are illustrated in Figure 2. The figure reveals that in both 1991 and 1992 the potency of downstream samples, both with and without S9 activation, was significantly greater than those collected upstream. In the presence of S9 activation, the potency of all samples was substantially reduced. Neither the upstream or the downstream BSED samples provided genotoxic extracts.

Following the 1991 investigations conducted at La Tuque, SPM and BSED samples were collected at 19 sites along the St. Lawrence and Saguenay rivers. Total regional, genotoxic loadings (in g BaP equivalents per day) at the sites investigated are provided in Table 1. Values range from less than 1 g at La Prairie to 31.1 kg at Pointe-aux-Trembles. Although we did not directly investigate effluents in the Beauport and Beaupré regions, chemical analyses detected known genotoxins in discharged effluents (EIS, 1992). Including the La Tuque samples described above, a total of 24 SPM and BSED samples from 22 sampling sites (21 sites in Figure 1 plus L. Memphremagog) were examined for genotoxicity¹. Figure 3 provides an example of SOS Chromotest concentration response plots obtained. The figure reveals that while the Jonquière SPM sample is more potent in the absence of S9 activation, the reverse is true for the BSED sample. This pattern was prevalent throughout the results. In addition, positive BSED samples rarely elicited SOS induction factors that exceed 1.5, while SPM samples often elicited induction factors above 2.0. In several cases maximum induction factors of SPM extracts exceeded 4.0.

Almost all the SPM extracts examined (21 of 22) elicited a significant positive response in the absence of S9 activation. In the presence of S9 activation, only 45% elicited

¹Due to an experimental error, results for SPM from Beauport and Beaupré cannot be used.

Figure 2. Genotoxic potency of SPM extracts from the St. Maurice river near La Tuque. The upstream site is located approximately 500 m above the final effluent outfall of a pulp and paper facility releasing a bleached Kraft mill effluent. The downstream site is located approximately 500 m below the effluent outfall. Samples were collected in June 1991 and again in June 1992. Error bars are one standard error of the mean SRIP. Where error bars are not shown they were too small. Asterisks indicate the results of SRIP comparisons. ** indicates significantly different at $\alpha = 0.01$. *** indicates significantly different at $\alpha = 0.001$. Comparisons were performed according to Zar (1984).

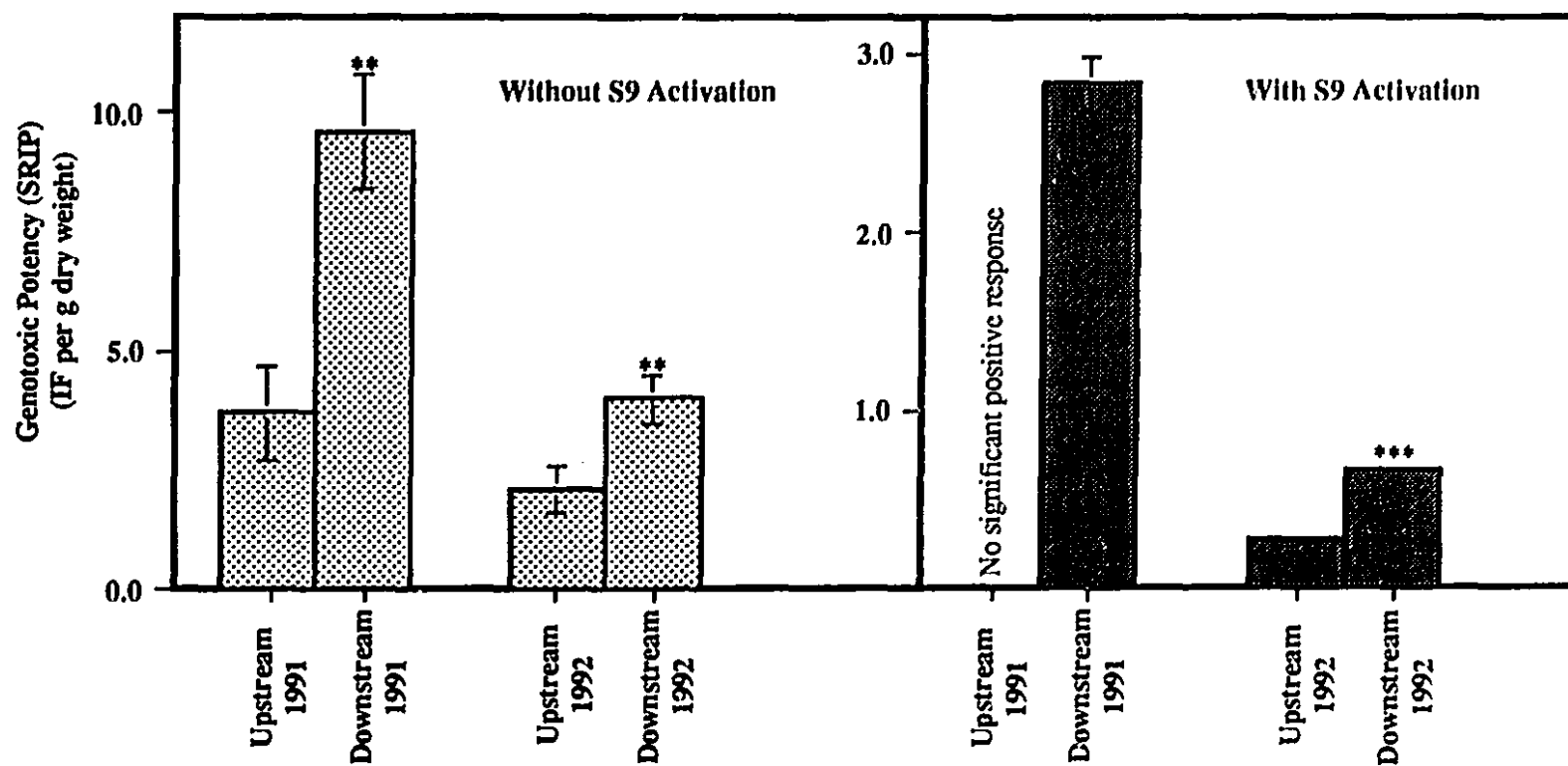
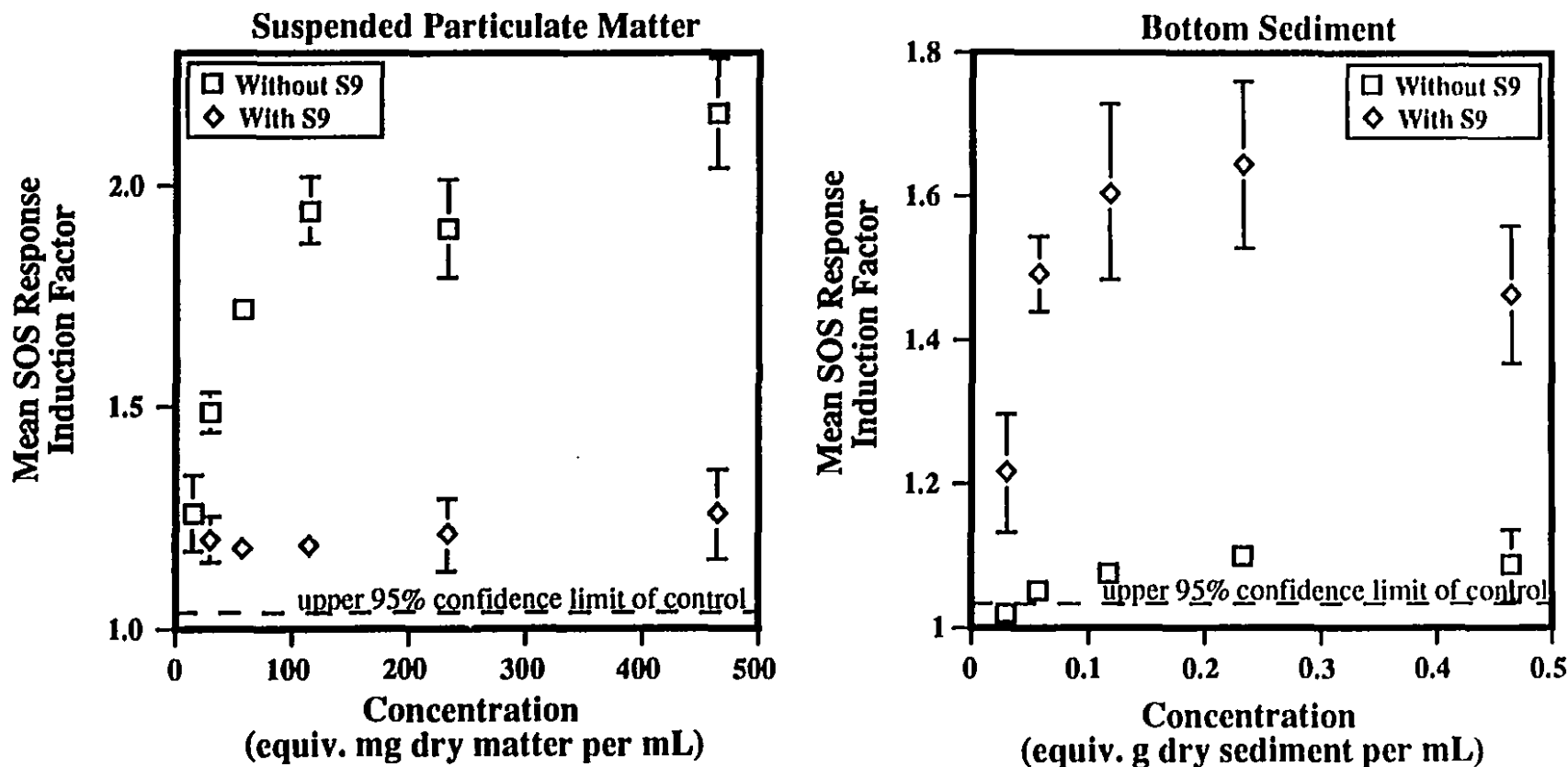


Figure 3. SOS Chromotest concentration response plots for suspended particulate matter and bottom sediment extracts from Jonquière (site 20). Induction factor values are means of duplicate samples. Error bars are one standard error of the mean. Where error bars are not shown they were smaller than the plotting symbol. Confidence limits on the controls which receive blank solvent were calculated according to Welsch et al. (1988).



a significant, positive response. The remaining samples either failed to elicit an SOS response (23%), elicited a marginal, positive response (23%) or elicited an erratic response (9%). Few BSED samples (12.5%) elicited a positive response in the absence of S9 activation. The remaining samples either elicited an erratic response (12.5%), or failed to elicit a positive response (75%). Although only 12.5% of the BSED samples elicited a positive response in the presence of S9 activation, an additional 25% of the samples elicited a marginal response. The remaining samples either failed to elicit an SOS response (50%) or elicited an erratic response (12.5%). Tables 4 and 5 provide a summary of MaxIF, SRIP and MDGC values for all positive, marginal or erratic SPM and BSED samples.

SRIP values for St. Lawrence and Saguenay river SPM samples ranged from a low of 1.32 IF per g at Tracy to a high of 133.7 IF per g at Boucherville. Ironically, the SRIP of the SPM sample from L. Memphremagog is several times that of the Boucherville sample. This is at least in part a consequence of the low concentration of particulate matter in L. Memphremagog. ANCOVA revealed a significant negative effect of particulate matter concentration and activation status on the SRIP of SPM extracts. The result obtained is summarized in the following model (model 1) ($r^2 = 0.70$, $n = 36$, F Ratio = 39.9, $p < 0.0001$, all effect significant at $p < 0.0001$):

$$\text{Log SRIP (River SPM)} = \begin{bmatrix} 1.98 \text{ (If S9 Not Present)} \\ 0.98 \text{ (If S9 Present)} \end{bmatrix} - 0.80 * \text{Log TSP} \quad (1)$$

Higher level effects were not significant. The ANCOVA result is illustrated in Figure 4. The model obtained is very similar to that identified in our previous analyses of suspended particulate matter in industrial effluents (White et al., 1995a). Statistical comparisons (Zar, 1984) did not reveal any significant difference between the coefficient of particle concentration in the above (model 1) and the corresponding coefficient in model 1 of White et al. (1995a). The ANCOVA result also indicates that the addition of S9 microsomal enzymes results in a decrease in potency of one order of magnitude. White et al. (1995a, 1995b) observed similar potency decreases in their examinations of industrial effluents.

Table 5 summarizes the results of bottom sediment extracts that elicited positive, marginal or erratic responses. Only 3 samples elicited a positive response in the absence of S9 activation, with an additional 3 sites eliciting marginal, erratic responses. A total of 9 sites elicited positive or marginal responses in the presence of S9 metabolic activation. With an additional 4 sites eliciting erratic responses. Table 6 summarizes the results of the PAH analyses. All of the sites that provided genotoxic samples, except for St. Romuald, contain

Table 4. Suspended particulate matter genotoxicity results. Samples not listed never elicited SOS induction factors that exceeded the solvent control.

Site	Activation	SOS Chromotest Result [†]	SRIP (IF g dry wt ⁻¹)	MDGC (mg dry wt.)	MaxIF
Beauharnois	N	POS	101.7	1.89	2.98
La Prairie	N	POS	35.20	4.04	2.53
Longueuil	N	POS	20.00	1.90	1.81
Montréal-East	N	POS	41.60	0.00190	5.00
Pointe-aux-Trembles	N	POS	8.890	NC*	2.48
Boucherville	N	POS	133.7	3.19	1.60
Varenes	N	POS	18.10	10.8	3.20
Contrecoeur	N	POS	55.12	1.72	1.98
Tracy	N	POS	1.316	18.5	1.28
Sorel	N	POS	50.40	6.58	1.41
La Tuque 1-1991	N	POS	3.700	NC	3.70
La Tuque 1-1992	N	POS	2.100	48.5	3.40
La Tuque 2-1991	N	POS	9.595	5.47	2.10
La Tuque 2-1992	N	POS	3.997	NC	4.38
Trois Rivières	N	POS	13.86	1.76	1.66
Bécancour	N	POS	26.78	3.51	1.75
Donnacoona	N	POS	3.534	9.37	1.36

Table 4 continued....

St. Romuald	N	POS	4.162	NC	3.30
Alma	N	POS	76.60	1.13	2.11
Jonquière	N	POS	17.10	7.53	2.16
Memphremagog	N	POS	587.0	0.97	1.20
Beauharnois	Y	POS	7.06	7.52	1.37
La Prairie	Y	MARG	1.80	44.7	1.08
Longueuil	Y	POS	1.16	12.1	1.31
Montréal-East	Y	POS	7.36	2.28	2.03
Boucherville	Y	M/Erratic	.	.	1.08
Varenes	Y	POS	4.61	8.00	1.45
Contrecoeur	Y	MARG	7.57	31.6	1.39
La Tuque 1-1992	Y	MARG	0.213	189.2	1.29
La Tuque 2-1991	Y	POS	2.48	8.68	1.29
La Tuque 2-1992	Y	POS	0.654	96.4	1.71
Bécancour	Y	MARG	2.60	84.9	1.16
Trois Rivières	Y	MARG	1.62	48.9	1.16
Donnacoona	Y	M/Erratic	.	.	1.05
St. Romuald	Y	POS	0.382	14.2	1.22
Alma	Y	POS	3.15	10.1	1.32
Jonquière	Y	POS	0.613	13.6	1.62
Memphremagog	Y	MARG	41.0	3.24	1.13

Table 4 continued.....

Solvent Blank	N/Y	NEG	-----	-----	1.04

†POS=Positive, MARG=Marginal, M/Erratic=Marginal/Erratic, NEG=Negative.

*NC=not calculated.

Table 5. Bottom sediment genotoxicity and PAH results. Samples not listed never elicited SOS induction factors that exceeded the solvent control.

Site	Activation	SOS Chromotest Result [†]	SRIP (IF g dry wt ⁻¹)	MDGC (g dry wt.)	MaxIF	Total PAH (ppb - dry wt)
Montreal-East	N	POS	1.68	0.073	1.21	23743.3
Varennes	N	M/Erratic	.	.	1.05	5336.1
St. Romuald	N	POS	0.263	.	1.11	59.8
Beauport	N	M/Erratic	.	.	1.04	48.0
Jonquière	N	POS	0.976	0.015	1.10	5589.8
La Baie	N	M/Erratic	.	.	1.03	335.2
Montreal-East	Y	POS	17.9	0.0064	1.44	23743.3
Longueuil	Y	M/Erratic	.	.	1.03	718.4
Boucherville	Y	MARG	3.35	0.038	1.16	1186.6
Varennes	Y	P/Erratic	.	.	1.16	5336.1
Contrecoeur	Y	M/Erratic	.	.	1.07	416.2
Trois Rivières	Y	MARG	1.87	0.067	1.17	748.0
Donncona	Y	MARG	0.650	0.26	1.03	107.3
St. Romuald	Y	MARG	0.228	0.30	1.18	59.8
Beauport	Y	MARG	0.110	0.51	1.04	480.0
Beaupré	Y	MARG	0.400	0.11	1.07	211.9

Table 5 continued.....

Jonquière	Y	POS	21.3	0.017	1.64	5589.8
La Baie	Y	POS	0.714	0.11	1.16	335.2
Memphremagog	Y/N	NEG	NA	NA	<1.00	42.0

†As in Table 4. P/Erratic = Positive/Erratic.

Figure 4. The effect of TSP (total suspended particulates in mg per L) and S9 activation on the genotoxic potency of SPM extracts. The fitted lines represent the ANCOVA model described in the text (model 1). The difference in intercept reflects the effect of S9 activation on genotoxic potency. Higher level effects were not significant at $\alpha = 0.05$.

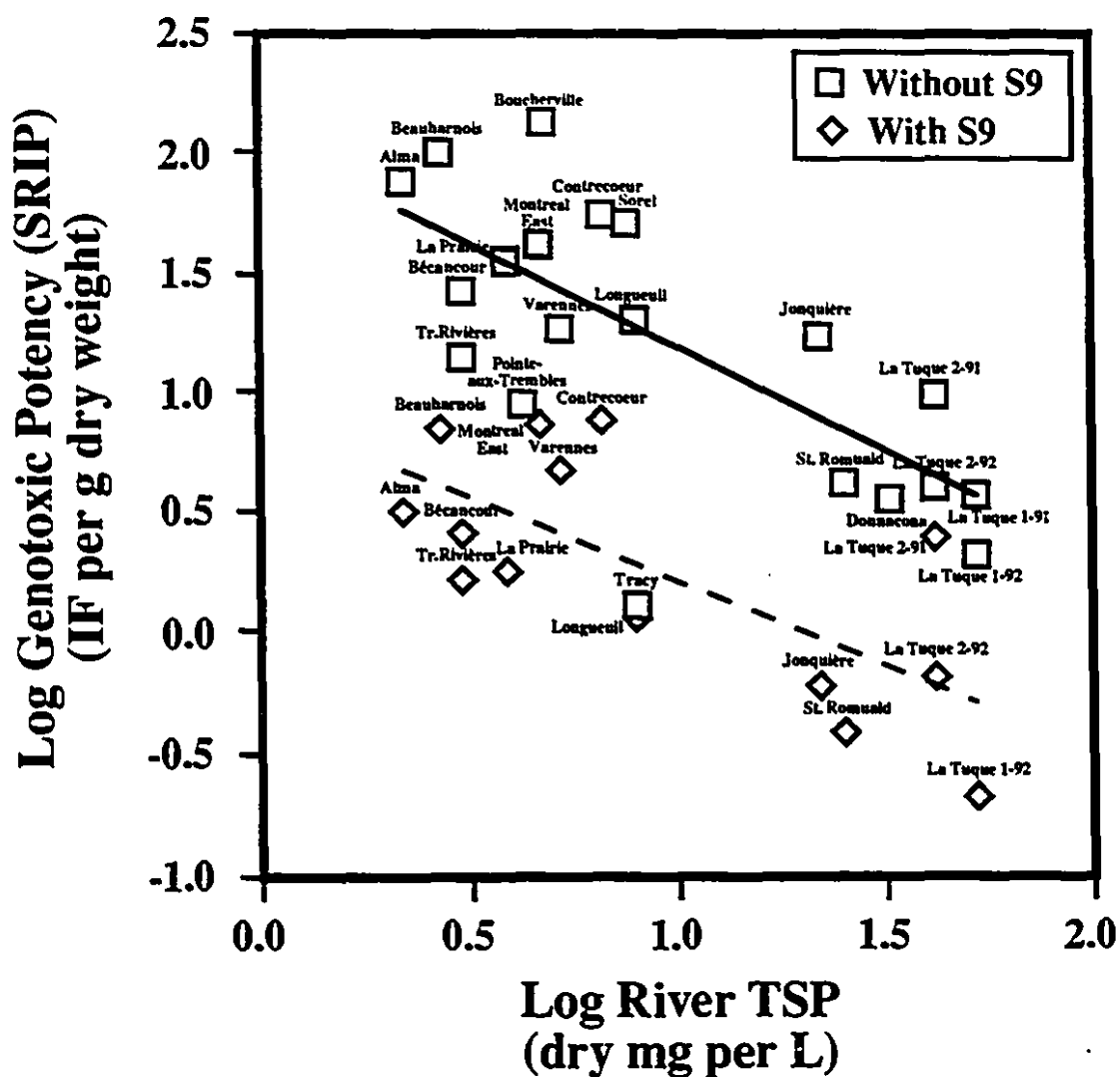


Table 6. Concentrations (ppb - dry weight) of identified genotoxic PAHs in bottom sediments.

Site	PHEN	ANTH	FLUOR	PYR	B(c)P	B(a)A	CHRY	B(b)kF	B(a)P	I(123cd)P	DB(ah)A	B(ghi)P	DB(ai)P	DB(ah)P	DB(al)P
Beauharnois	43.2	10.8	123.7	149.2	0.0	84.4	314.2	314.2	117.8	72.7	23.6	94.3	0.0	0.0	23.6
Longueuil	54.0	6.8	150.0	110.0	0.0	36.0	84.0	116.0	50.0	32.0	8.2	38.0	6.4	7.0	10.6
Montréal-East	1960.8	2574.8	3168.9	3565.1	376.3	1426.0	4357.3	2376.7	455.5	614.0	515.0	713.0	435.7	376.3	356.5
P. aux-Trembles	46.0	22.0	50.0	38.0	0.0	14.6	54.0	44.0	24.0	8.0	3.2	8.2	0.0	0.8	2.2
Boucherville	109.1	35.7	218.2	152.7	0.0	39.7	136.8	156.7	93.2	49.6	12.7	45.6	0.0	11.1	18.6
Varenes	2193.0	398.7	458.5	1236.0	49.8	49.8	187.4	23.9	16.2	2.6	1.0	4.0	0.0	1.2	2.0
Contrecoeur	27.9	6.8	67.8	57.8	0.0	17.6	57.8	71.8	29.9	19.1	5.8	23.9	0.0	0.0	7.8
Tr. Rivières	10.0	34.0	122.0	140.0	8.2	34.0	80.0	78.0	42.0	26.0	5.4	26.0	9.6	6.4	12.2
Donnacona	7.4	1.8	18.6	17.2	0.0	3.2	30.0	13.8	5.0	3.0	0.8	3.6	0.0	1.4	0.8
St. Romuald	2.8	1.2	10.6	10.0	0.4	1.8	11.2	9.2	3.6	2.2	0.4	2.4	1.2	0.6	1.6
Beauport	2.8	0.8	8.2	7.4	0.0	0.8	9.4	8.2	4.2	2.4	0.4	2.6	0.0	0.0	0.4
Beaupré	10.5	5.0	27.8	27.8	0.0	4.4	41.8	45.7	19.9	10.3	4.0	12.9	0.0	0.0	0.0
Alma	55.2	7.7	98.6	78.9	0.0	27.6	76.9	75.0	33.5	17.2	3.4	18.7	0.0	1.4	6.1
Jonquière	222.6	101.2	708.1	581.7	40.5	250.4	1011.6	1011.6	43.0	278.2	123.9	354.1	141.6	91.1	169.4
La Baie	40.0	17.4	64.0	58.0	3.0	12.2	38.0	34.0	20.0	10.8	3.4	12.6	4.2	2.8	6.8
Memphremagog	1.8	0.3	5.5	5.6	0.0	1.8	4.6	10.9	2.5	4.8	1.2	4.6	0.3	0.3	1.0
Solvent Blank	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

more than 100 ppb of total PAHs, with three of genotoxic samples containing more than 1 ppm.

Many studies have demonstrated that the sorption of organic substances to particulate matter is directly related to the carbon content of the particulate matter (e.g. Karickhoff et al., 1979; Swarzenbach and Westall, 1980; Mean et al., 1980; Choi and Chen, 1976; Prahl and Carpenter, 1983). We investigated the effect of sediment organic carbon content on genotoxic potency and PAH contamination. The results obtained indicate that both variables are related to sediment organic carbon content. These results are illustrated in Figure 5. The figure reveals that organic carbon content has the identical effect on PAH concentration (Figure 5a) and genotoxic potency (Figure 5b), suggesting similar physical-chemical properties. The darkened symbols on the plots (Jonquière, Montreal-East and Varennes) do not appear to follow the general trend and were considered outliers. If the outliers are excluded, 75% of the variation in PAH concentration can be explained by sediment carbon content (Figure 5b). Regression analyses revealed the following model (model 2) ($r^2 = 0.75$, $n = 12$, F Ratio = 30.3, $p < 0.001$):

$$\text{Log PAH (River BSED)} = -1.04 + 1.06 * \text{Log LOI} \quad (2)$$

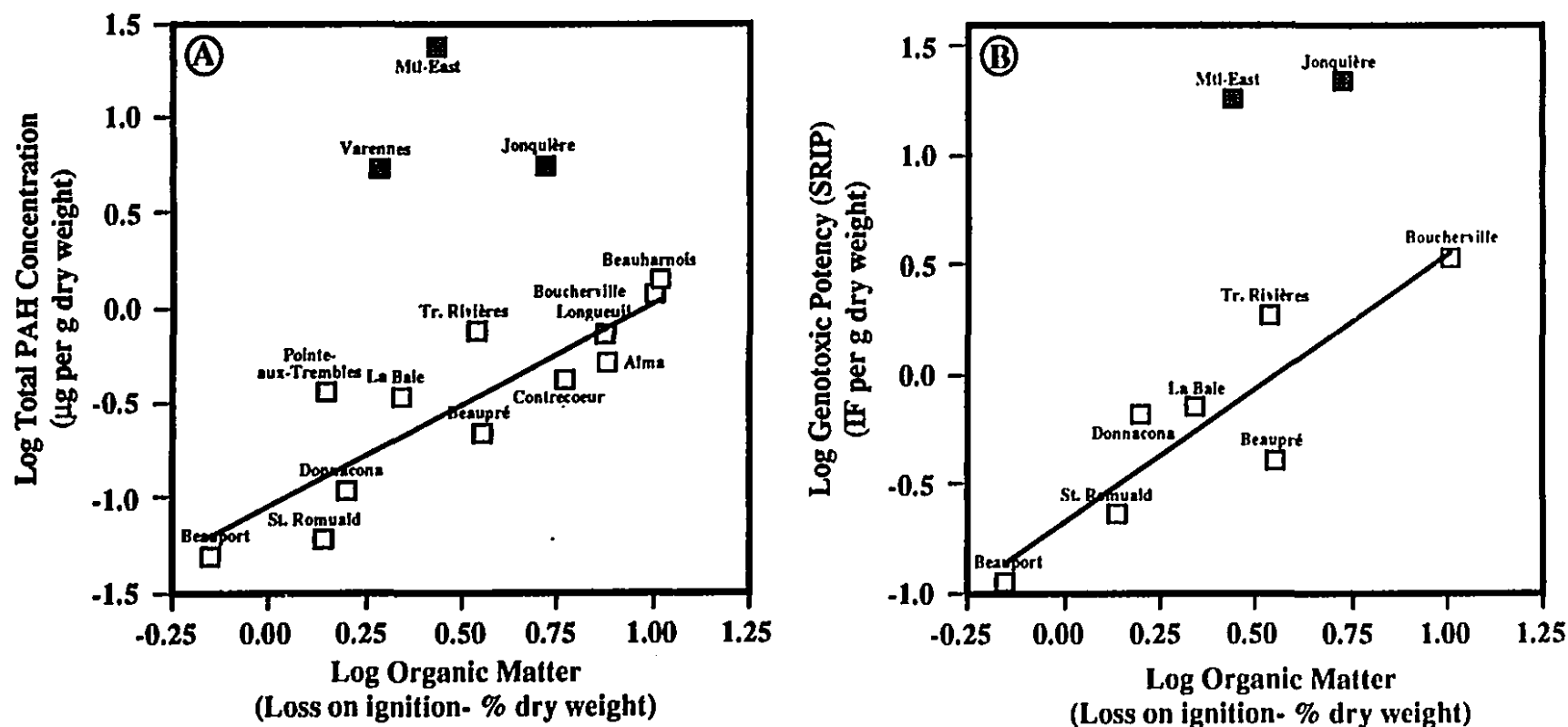
In a similar manner, 78% of the variation in SRIP can be explained by sediment carbon content. Regression analysis revealed the following model (model 3) ($r^2 = 0.78$, $n = 7$, F Ratio = 17.8, $p < 0.01$):

$$\text{Log SRIP (River BSED)} = -0.67 + 1.22 * \text{Log LOI} \quad (3)$$

Further analyses indicated that the coefficients of LOI in models 2 and 3 are not significantly different. In addition, neither coefficient is significantly different from 1.00. Although these results indicate that genotoxins and PAHs in bottom sediments likely have similar physical-chemical properties, the results do not indicate whether the detected PAHs can account for the observed genotoxic response. In an effort to address this question, both the PAH values and the genotoxicity values were converted to a common unit- $\mu\text{g BaP}$ equivalents per g dry weight (i.e. ppm BaP equivalents). Bioassay-derived concentrations of BaP equivalents were calculated as the ratio of the BaP MDGC value ($0.0757 \mu\text{g per assay mL}^1$) to the sample MDGC value (g dry weight per assay mL). PAH-derived

¹mean of 40 separate measurements made in our laboratory.

Figure 5. The effect of sediment organic matter (as LOI in % dry weight) on the PAH concentration and genotoxic potency of bottom sediments. A- The effect of organic content on the total concentration of 21 PAHs. The fitted line represents the regression function described in the text (model 2). The darkened symbols were not included in the calculation of the regression function. B- The effect of organic content on the genotoxic potency of bottom sediment samples. The fitted line represents the regression function described in the text (model 3). Again, the darkened symbols were not included in the calculation of the regression function.

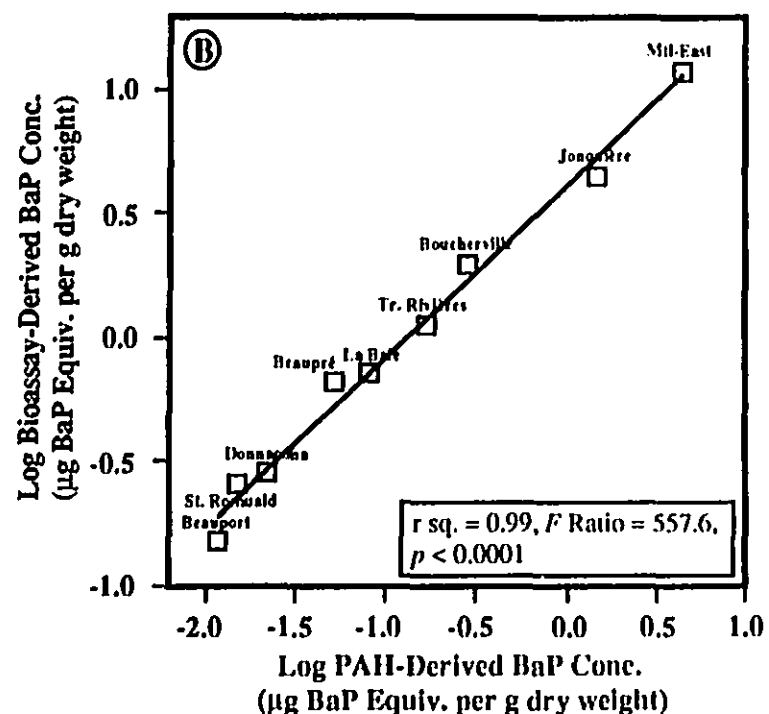
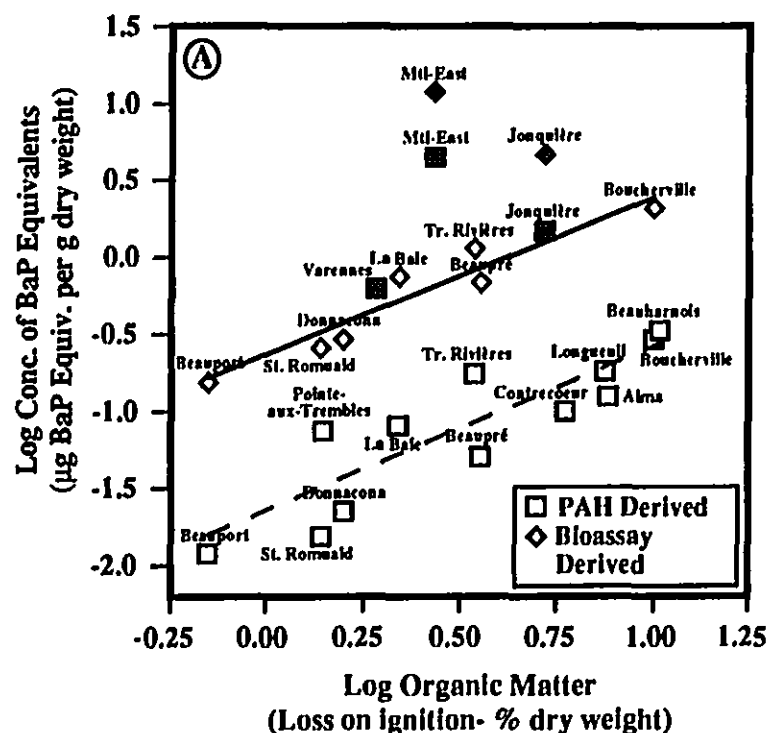


concentrations of BaP equivalents are the sums of the PAH concentrations in Table 6 multiplied by their respective genotoxic equivalency factors listed in Table 3. Bioassay-derived concentrations of BaP equivalents obtained here (geometric mean = 0.52 ppm BaP Equiv.) are similar to those calculated from *Salmonella* mutagenicity results of Tama river sediment extracts (0.32 to 3.6 ppm BaP equiv., Suzuki et al., 1982). Figure 6a illustrates the effect of sediment organic matter content on bioassay-derived and PAH-derived concentrations of BaP equivalents. The organic matter effect shown is identical to that shown in Figure 5. However in Figure 6a it becomes apparent that the bioassay derived concentrations of BaP equivalents are approximately one order of magnitude higher than the corresponding PAH-derived values. Figure 6b illustrates that there is an extremely strong relationship between the concentration of bioassay-derived BaP equivalents and the corresponding PAH-derived values. Although preliminary analyses revealed an empirical relationship between BSED SRIP and total PAH ($r^2 = 0.89$, $p < 0.002$), the conversion of PAH values to BaP equivalents resulted in a 10% increase in r^2 . The relationship obtained is summarized in the following regression model (model 4) ($r^2 = 0.99$, $n = 9$, F Ratio = 557.6, $p < 0.0001$):

$$\text{Log BaP Equiv (Bioassay derived)} = 0.61 + 0.69 * \text{Log BaP Equiv (PAH derived)} \quad (4)$$

It is interesting to note that several sediment samples which failed to elicit a positive, genotoxic response are more contaminated with PAHs than some of the positive or marginal samples. For example, the PAH level at Varennes is an order of magnitude higher than that of genotoxic samples from La Baie, St. Romuald, Donnacona, Beauport, Contrecoeur and Beaupré. The Varennes BSED extract elicited a response (+S9) that exceeded the control at four concentrations. However, the erratic nature of the response did not permit SRIP and MDGC values to be calculated. Samples from Longueuil and Contrecoeur also elicited erratic responses (see Table 5). Expressing PAH concentrations as BaP equivalents reveals that sediments from Contrecoeur, Longueuil and Varennes should be more genotoxic than those from La Baie, Beauport, Beaupré and Donnacona. In addition, although samples from Alma, Beauharnois and Pointe-aux-Trembles failed to elicit positive or marginal responses, they contain higher concentrations of PAH-derived BaP equivalents than genotoxic samples from Donnacona, Beauport and Beaupré. We investigated the possibility that sediments from some contaminated sites contained toxic substances that interfere with SOS Chromotest performance. Alkaline phosphatase activity values indicate that no BSED samples elicited severe bacteriostatic effects.

Figure 6. A- The effect of sediment organic matter on the concentration of PAH-derived and bioassay-derived BaP equivalents. Fitted lines represent least squares regression functions. Darkened symbols were considered outliers and not included in the regression calculations. The dashed line represents the regression function for BaP equivalents derived from PAH analyses ($\text{Log BaP Equiv} - \text{PAH derived} = -1.68 + 0.96 \cdot \text{Log LOI}$, $r^2 = 0.78$, $n = 12$, $F \text{ Ratio} = 35.6$, $p < 0.0001$). The solid line represents the fitted regression function for BaP equivalents calculated from SOS Chromotest analyses ($\text{Log BaP Equiv} - \text{Bioassay derived} = -0.66 + 1.02 \cdot \text{Log LOI}$, $r^2 = 0.91$, $n = 7$, $F \text{ Ratio} = 53.4$, $p < 0.001$). Post-hoc comparisons did not reveal a significant difference in slopes. The difference in intercept indicates that concentrations of bioassay-derived BaP equivalents are one order of magnitude greater than those derived from PAH analyses. **B-** The relationship between the concentration of bioassay-derived BaP equivalents and the concentration of PAH-derived BaP equivalents. The plotted line represents the regression function described in the text (model 4).



The results presented in Tables 4 and 5 indicate that the potencies of SPM samples are approximately an order of magnitude higher than that of BSED samples. However, this is not a valid comparison since the bottom sediments are diluted with large particles that have a very low affinity for organic substances. Choi and Chen (1976), Karickhoff et al. (1979) and Frahl and Carpenter (1983) demonstrated that sand is a considerably less effective sorbent than smaller particulate matter. Granulometric measurements on all genotoxic sediment samples (see Table 2) were used to correct SRIP values for the proportion of the sample composed of inorganic material $> 63 \mu\text{m}$. Table 7 provides a comparison of total SRIP values for BSED and SPM samples. Total SRIP is defined as the sum of the direct-acting and S9 activated results, when S9 activation increased the potency of the sample. The results reveal that at three sites (Montreal-East, Jonquière and St. Romuald) the corrected bottom sediment SRIPs are greater than the corresponding SPM SRIPs. At Trois Rivières, Donnacona and Boucherville the corrected BSED SRIP values are lower than the corresponding SPM values.

It is interesting to note that 4 of the 5 regions which provided potent SPM samples (Alma, Beauharnois, Contrecoeur, and Trois Rivières; see Tables 1 and 4) receive wastes from metal founding industries and aluminum refineries. Both of these industries employ high temperature combustion processes known to produce genotoxic PAHs (IARC, 1984). Montreal-East also provided a potent SPM sample. This site receives wastes from two large petroleum refineries. Petroleum refining has also been identified by the IARC as an industry that produces and releases genotoxic substances (IARC, 1989a). In addition, White et al. (1995a) frequently classified emissions from petroleum refining, metal founding and Al refining as *high potency* wastes. Further investigations revealed an empirical relationship between the genotoxic potency of downstream SPM samples and the regional, weighted average potency of industrial effluent particulate matter. This relationship is illustrated in Figure 7. The results obtained reveal a significant relationship across 11 of the 14 sites for which data are available. Least-squares linear regression provided the following result (model 5) ($r^2 = 0.87$, $n = 11$, F Ratio = 60.2, $p < 0.0001$):

$$\text{Log SRIP (River SPM)} = 0.031 + 0.53 * \text{Log Mean SRIP (Effluent SPM)} \quad (5)$$

Both SRIP values are expressed in IF units per equivalent g of dry particulate matter. The intercept (0.031) is not significantly different from zero. The model does not include data from Sorel, La Prairie or Boucherville. These sites provided samples that are more genotoxic than known industrial discharges would suggest.

We also investigated empirical relationships between BSED genotoxicity results and

Table 7. Comparison of total genotoxic potency values for SPM and BSED extracts.

Site	Total SPM SRIP [†] (IF per g dry wt.)	Total BSED SRIP (IF per g dry wt.)
Montreal-East	41.6 - N	310.4 - N/Y*
Jonquière	17.1 - N	36.2 - N/Y
St. Romuald	4.2 - N	48.1 - N/Y
Boucherville	133.7 - N	12.7 - Y
Donnacoona	3.5 - N	2.4 - Y
Trois Rivières	13.9 - N	10.2 - Y

[†]Total SRIP is the sum of SRIP without S9 and SRIP with S9, if SRIP with S9 > SRIP without S9.

*Letters indicate whether the values include with S9 values, without S9 values or both.

regional industrial loadings. Figure 8a reveals the relationship between BSED genotoxicity (+S9) and the maximum, regional loading of genotoxins adsorbed to effluent particulate matter. Similar to the results already shown in Figure 7, Figure 8 suggests that the Boucherville site is receiving substantial, unknown genotoxic discharges. Excluding the Boucherville observation, linear regression revealed the following model (model 6) ($r^2 = 0.86$, $n = 6$, F Ratio = 25.1, $p < 0.008$):

$$\text{Log SRIP (Bottom Seds +S9)} = -7.294 + 0.92 * \text{Log Max. Effluent Loading (SPM)} \quad (6)$$

Figure 8b reveals the relationship between BSED genotoxicity (+S9) and the maximum, regional industrial loading of dissolved base/neutral compounds. Again, excluding the results obtained at Boucherville, linear regression revealed the following model (model 7) ($r^2 = 0.86$, $n = 6$, F Ratio = 18.0, $p < 0.02$):

$$\text{Log SRIP (Bott. Seds +S9)} = -5.04 + 0.64 * \text{Log Max. Effluent Loading (Base/Neutral)} \quad (7)$$

Figure 7. The relationship between the genotoxic potency of river SPM samples and the regional, weighted average potency of particulate emissions from local industries. Genotoxic potency values of suspended particulate matter from the industries in a given region (see White et al., 1995b) were weighted by their relative contribution to the total regional loading of particulate matter in g per day. The fitted line is the least squares regression fit described in the text (model 5). Darkened points were not included in the calculation of the regression function. If all data are included the relationship is no longer significant ($r^2 = 0.19$, $p = 0.12$).

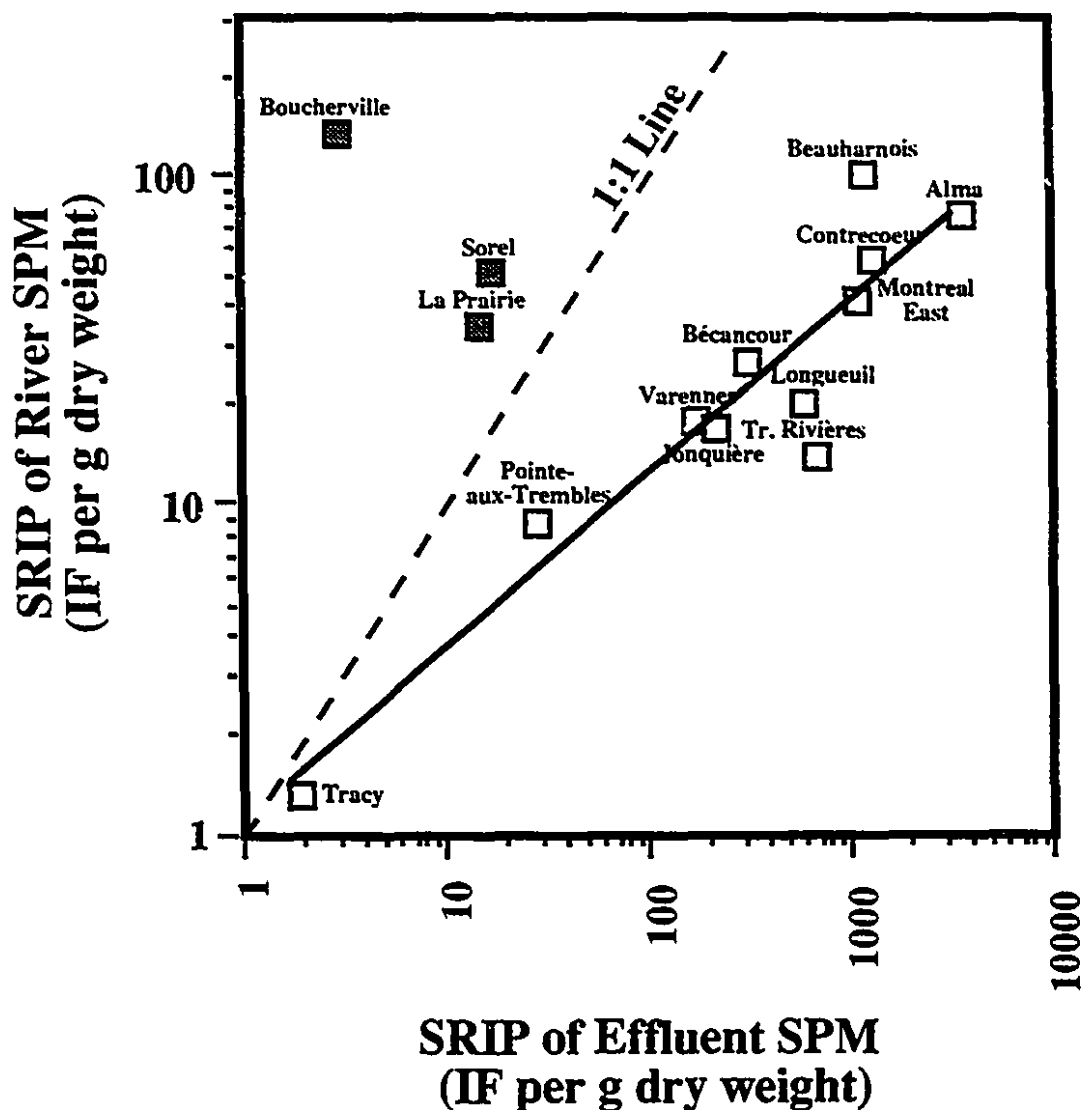
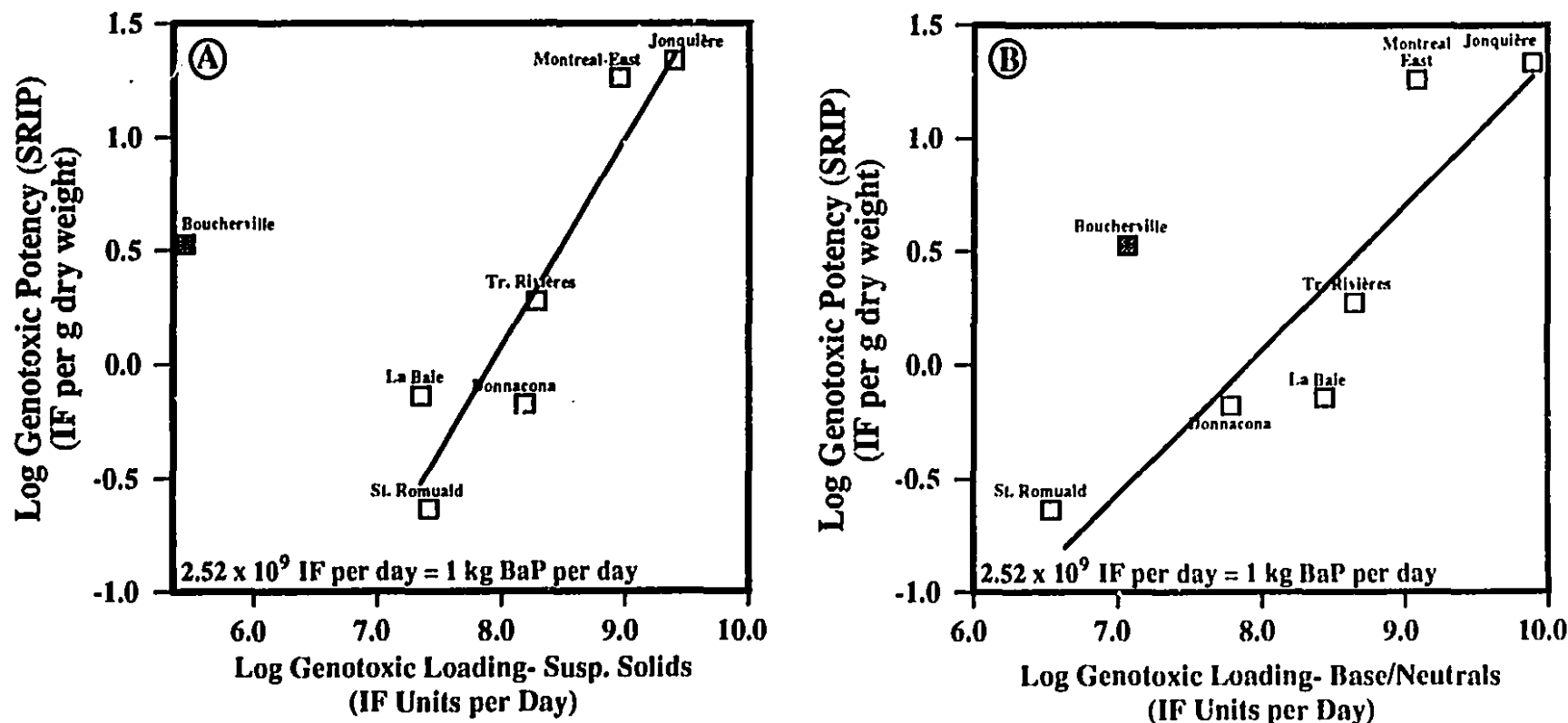


Figure 8. The relationships between the genotoxic potency of bottom sediment extracts and regional industrial loadings. **A-** The relationship between bottom sediment SRIP (+S9) and the regional industrial loading of substances adsorbed to effluent suspended particulate matter. The fitted line represents the regression function described in the text (model 6). **B-** The relationship between bottom sediment SRIP (+S9) and the regional industrial loading of dissolved base/neutral substances. The fitted line represents the regression function described in the text (model 7). In both plots the darkened symbol was not included in the regression calculations. Loadings are maximum daily values calculated from the results of White et al. (1995b). The conversion factor provided can be used to covert loading values to their genotoxic BaP equivalent.



DISCUSSION

The primary goal of this work was to investigate the presence and potency of organic genotoxins sorbed to particulate material in the St. Lawrence river system. The results presented in Table 4 indicate that all sites except La Baie provided genotoxic SPM samples. SRIP values obtained are generally between 1 and 100 IF per g dry weight (≈ 0.01 -0.6 IF per L). Few researchers have investigated the genotoxicity or mutagenicity of surface water suspended particulate matter. Durant et al. (1994) obtained significant genotoxic responses on a human B cell mutation assay from 130 mL equivalent of surface water per assay mL. The results obtained here (Table 4) indicate that concentrations between 0.4 mL equivalent per assay mL (Montreal-East) and 924 mL equivalents per assay mL (La Tuque 1) are sufficient to produce a significant genotoxic response on the SOS Chromotest. Grifoll et al. (1992) used the Ames/*Salmonella* mutagenicity assay (Ames et al., 1973; Ames et al., 1975) to investigate the mutagenicity of DMSO extracts of particulate matter from the Llobregat river (Spain). Grabow et al. (1980) measured the Ames/*Salmonella* mutagenicity of dichloromethane extracts of suspended particulates from the Vaal River. Although neither of these studies used the SOS Chromotest, the empirical models developed by White and Rasmussen (1995) can be used to predict the *Salmonella* mutagenicity of the SPM samples examined in this study. The geometric mean potency of SPM extracts examined here (in IF units per equivalent L) is equivalent to ≈ 14 *Salmonella* TA98 revertents per L (no S9). This value is similar to that obtained by Grabow et al. (1980, ≈ 28 revertents per L on TA98¹), but far lower than that obtained by Grifoll et al. (1992, ≈ 7 -13 rev per mL). The high potency of the Llobregat river particulate matter samples is likely due to high levels of suspended solids and high loadings of industrial and municipal wastes. The Llobregat is a small river (discharge $< 10 \text{ m}^3 \text{ sec}^{-1}$) located in a heavily populated, industrialized basin. It receives water that is grossly polluted with industrial and sanitary wastes that receive virtually no treatment (Prat et al., 1984).

Although several researchers have investigated genotoxins in sediments, relatively few have employed the SOS Chromotest. Langevin et al. (1992), Dutka et al. (1987) and Xu et al. (1987) used the SOS Chromotest to investigate the genotoxicity of sediments from the St. Lawrence river, the Detroit river and ponds in Prince Edward Island. The results obtained in those studies (≈ 1 - 25 IF per g dry wt.) are similar to those obtained in this study (geometric mean SRIP of BSED = 1.3 IF per g). Again, the models of White and Rasmussen (1995) can be used to permit a comparison of the results obtained here with the

¹All *Salmonella* mutagenic potency values were adjusted for variations in top agar volume as described in White and Rasmussen (1995).

large number of studies that employed the *Salmonella* mutagenicity test to examine contaminated sediments. The geometric mean SRIP value of BSEDs (with S9) is equivalent to ≈ 700 *Salmonella* TA98 revertents per g dry wt (≈ 2600 revertents per mg extractable matter). This value is higher than those obtained by Grifoll et al. (1990) and Sato et al. (1983) in studies of sediments from the Besos (≈ 400 TA98 revertents per mg residue with S9) and Nagara (≈ 200 TA100 revertents per g dry wt. with S9) rivers. However, the values are lower than those obtained for severely contaminated sediments from Hamilton Harbour (Lake Ontario) and the Black River (Ohio). West et al. (1988) described potency values $> 10^5$ TA98 revertents per mg residue in a PAH-containing fraction of Black River sediment. Balch et al. (1995) measured values > 5000 TA98 revertents per g dry wt for Hamilton Harbour sediment. Chemical analyses demonstrated that PAH levels in Hamilton Harbour sediments exceeds several hundred ppm (dry wt.).

The results obtained at La Tuque, Quebec (Figure 2) suggests that the bleached Kraft mill is releasing particle bound genotoxic material into the St. Maurice river. In both 1991 and 1992 the downstream samples yielded extracts that were significantly more potent than the corresponding upstream samples. However, in both sampling years, the upstream SPM samples were also genotoxic. Upstream genotoxicity is likely due to the presence of resin acids from softwood lumber. Several studies have documented the genotoxicity of natural resin acids (Nestmann et al., 1979; Kringstad and Lindström, 1984). Visual examination of the upstream area confirmed the presence of large amounts of bark and wood fragments released during lumber transportation. The increase in potency at the downstream site is likely due to the production and release of chlorinated organics. Potent genotoxins isolated from softwood Kraft mill effluents include chlorinated organics released in spent chlorination liquors (Kringstad and Lindström, 1984; Kringstad et al., 1984; Holmbom et al., 1981; Bjørseth et al., 1979). Figure 2 also indicates that the 1992 samples are substantially less potent than the 1991 samples. Downing (1993) found similar changes in biochemical markers of fish stress. The decline was attributed to a large decrease in the emission of adsorbable halogenated organics. The results also indicate a large decrease in potency when SPM extracts were tested in the presence of S9. Similar decreases have been observed for pulp and paper mill effluent samples (Bjørseth et al., 1979; McGeorge et al., 1985). Despite evidence that mutagens in bleached Kraft mill effluents can accumulate in coastal sediments (Kinae et al., 1981), we did not detect any genotoxic activity in bottom sediments from La Tuque.

S9 microsomal enzymes were added to the assay mixture to permit the detection of pro-genotoxic substances that are active DNA damaging agents only after metabolic activation. However, for the SPM extracts, the potency of all samples was reduced when

tested in the presence of S9 enzymes. Similar decreases in the potency of complex environmental samples have been observed by many researchers (e.g. White et al., 1995a; McGeorge et al., 1985; Savard et al., 1992; Courtois et al., 1988; Courtois et al., 1992). Courtois et al. (1988 and 1992) attributed the reduction to a physical interference of the S9 proteins with the action of direct acting substances. Although many researchers have observed such decreases, there is little agreement as to whether surface water genotoxins are primarily direct acting or require S9 metabolic activation. The results presented here and elsewhere (Langevin et al., 1992; Grabow et al., 1981; Grabow et al., 1980; Grifoll et al., 1992) suggest that surface water genotoxins are primarily direct-acting. Langevin et al. (1992) found that S9 reduced the potency of St. Lawrence river DCM extracts. Grabow et al. (1981) and Grabow et al. (1980) found that S9 rarely increased the mutagenicity of Vaal river DCM extracts. Grifoll et al. (1992) found that S9 had little effect on the potency of DMSO extracts of particulate matter from the Llobregat river. On the contrary, a large number of studies have indicated that S9 dramatically increases the potency of surface water extracts (e.g. Sloof and van Kreijl, 1982; Dutka et al., 1981; Paola et al., 1993; Maruoka and Yamanaka, 1983; Hendriks et al., 1994; Sakamoto and Hayatsu, 1990; Maruoka et al., 1985). The vast majority of these studies used XAD resin adsorption, rather than liquid-liquid extraction, for extract preparation. Thus, the effect of S9 may be related to the method used for extraction/concentration, with DCM extraction being more effective than resin adsorption at isolating direct acting genotoxins from water. This supposition is supported by the works of several researchers. Grabow et al. (1981) revealed that the quantity of DCM extractable material is frequently greater than that obtained via XAD adsorption. Jolley (1981) and Junk et al. (1974) indicate that XAD methods may not provide efficient recoveries of certain classes of trace organics. Waters et al. (1989) found little difference in the affinity of XAD resins and *blue cotton* (Hayatsu, 1990) for surface water mutagens. Blue cotton is known to adsorb highly lipophilic, aromatic substances with 3 or more fused rings (Hayatsu, 1990). In contrast, dichloromethane has been called the best solvent for extraction of a wide range of trace organics in water (Hunchak and Suffet, 1987).

In contrast to the SPM results, the BSED samples were almost always more potent in the presence of S9. Despite substantial industrial loadings of direct acting substances sorbed to particulate matter, and the presence of direct acting activity in the water column, only three BSED samples (Montreal-East, St. Romuald, Jonquière) contained detectable levels of direct-acting substances. Since bottom sediments contain older material removed from the water column (Knezovich et al., 1987; Wetzel and Likens), the results suggest that direct acting substances are fairly readily degraded in the downstream ecosystem. This

may not be surprising since direct acting genotoxins are unstable electrophiles that will readily react with any available nucleophile (Albertini and Robison, 1991). Many other researchers have also noted that bottom sediment extracts frequently require S9 activation (e.g. Langevin et al., 1992; Ho and Quinn, 1993; Durant et al., 1992; Maccubbin and Ersing, 1991; Grifoll et al., 1988; Hashizume et al., 1992; Suzuki et al., 1983; Marvin et al., 1994; Balch et al., 1995). In fact, with the exception of Samoiloff et al. (1983) and Oishi and Takahashi (1987), few published studies have ever described direct acting bottom sediment extracts. Therefore, in contrast to direct acting substances, pro-genotoxins appear to be less readily degraded. Several studies have confirmed that pro-genotoxins such as unsubstituted aromatic hydrocarbons with 3 or more fused rings are relatively resistant to degradation and accumulate in sedimented particulate material (e.g. Smith and Levy, 1990; Balch et al., 1995; Malins et al., 1984; Gardner et al., 1991; Prahl and Carpenter, 1983).

The results presented in Figure 4 and model 1 reveal a negative effect of SPM concentration on the SRIP of SPM samples. This indicates that there is a large excess of suspended particulate matter relative to the total quantity of adsorbable genotoxins. The relatively high concentrations of particulate matter serves to dilute the pool of adsorbed genotoxins and reduce genotoxic potency values (IF Units per equivalent dry weight of particulate matter). This result is almost identical to that obtained in our earlier investigations of industrial effluents (White et al., 1995b).

The results presented in Figures 5 and models 2 and 3 indicate that the concentrations of PAHs and genotoxins in bottom sediment samples are linearly related to organic matter concentration (log-log regression coefficient not different from 1.0). This is similar to the results of Brown and Flagg (1981), Karickhoff et al. (1979), Choi and Chen (1976) and Means et al. (1980). However, the results also indicate that sediments from Montreal-East, Jonquière and Varennes contain more PAHs and genotoxins than the organic matter content would predict. As already mentioned (see Means et al., 1980; Karickhoff et al., 1979; Brown and Flagg, 1981; Choi and Chen, 1976; Swarzenbach and Westall, 1981; White et al., 1995a) sorption is primarily determined by solute K_{ow} and sorbent organic matter content. However, Karickhoff et al. (1979), Swarzenbach and Westall (1981) and Means et al. (1980) also indicated that 18-24 hours is required to establish sorption equilibrium. Therefore, deviation from the identified trend may be due to differences in the physical-chemical properties of contaminants in samples from these sites, or a lack of equilibrium between dissolved and particulate phases. Industries located at Jonquière (Al refineries) and Montreal-East (petroleum refineries) are known to produce and release high molecular weight aromatic and aliphatic hydrocarbons (IARC, 1984; IARC, 1989) that

would be expected to have very high K_{ow} 's (Mackay et al., 1992). In addition, the river current ($\approx 0.5 \text{ m sec}^{-1}$) and the distance of the sampling sites from the presumed source(s) indicates that the time between discharge and sample collection ($\approx 10 \text{ min}$) may not be sufficient to establish equilibrium partitioning.

Conversion of both genotoxicity and PAH values to a common unit simplifies comparisons between the two. Due to the difficulty in predicting the effects of complex chemical mixtures on exposed organisms, conversions of chemical measurements to biologically meaningful equivalents has become increasingly popular (Giesy et al., 1994; van den Heuvel et al., 1994). Although the results revealed a significant relationship between bottom sediment genotoxicity and total PAH concentration, conversion of the PAH values to their BaP equivalence resulted in a 10% increase in r^2 (see model 4). The results presented in Figure 6a indicate that PAH-derived concentrations of BaP equivalents are approximately one order of magnitude below those of bioassay-derived BaP equivalents. This suggests that the measured PAHs only account for one-tenth of the genotoxicity detected in the BSED extracts. Despite this discrepancy, Figure 6b indicates that there is an excellent relationship between the concentration of PAH-derived BaP equivalents and the concentration of bioassay-derived BaP equivalents. Thus, the putative genotoxins include the PAHs listed in Tables 3 and 6 as well as other unidentified substances that have similar physical-chemical properties. Several studies have confirmed that in addition to the PAHs listed in Table 2, genotoxic sediments contain a variety of genotoxic PAHs and PAH derivatives that are not usually detected by routine chemical analyses (Marvin et al., 1993; Balch et al., 1995; Ho and Quinn, 1993; West et al., 1988).

The major goal of many studies has been to document the (geno)toxicity of environmental samples from sites renowned for their severe contamination (e.g. Grifoll et al., 1988; Dutka et al., 1987; Sato et al., 1983; Maruoka and Yamanaka, 1983; Sloof and van Kreijl, 1982; Dutka and Switzer-House, 1978; Grabow et al., 1980). Knowledge about the post-emission behaviour of complex, genotoxic industrial emissions is extremely limited. Although previous investigations have revealed genotoxic activity in industrial effluents (see Houk, 1992 for review), surface waters and sediments (see Stahl, 1991 for review), and aquatic biota (White et al., 1995d), few efforts have been made to establish empirical or mechanistic links between field observations and effluent measurements. Upon emission, industrial genotoxins will be rapidly diluted with the water of the receiving system. The rate at which this dilution occurs is a complex function of pipe depth, effluent buoyancy and river current (Tsanis and Valeo, 1994). While detailed examinations of effluent dispersion is beyond the scope of this study, we were able to investigate empirical relationships between the field results and previously published effluent results (White et

al. 1995b). The results presented in Figure 7 revealed that for 11 sites, the SRIP of river SPM samples is empirically related to the regional weighted average potency of effluent particulate matter. The results suggest that the field samples are a diluted version of the effluent particulate matter. The regression coefficient of model 5 is significantly less than 1.0 and not significantly different from 0.5. Therefore, the genotoxic potency of river SPM can be predicted by the square root of the weighted average effluent potency. Lack of knowledge about the potency of incoming solids and the rate at which ambient water and its associated particulate matter are being entrained into the effluents prevented us from performing more detailed mass balance calculations. Three sites in Figure 7 did not fit this general trend. These sites either experience less dilution at the point of sampling or receive discharges that were not included in our analyses. All three of the outliers lie above the 1:1 line. Therefore, at the point of sampling, the SPM sample was more genotoxic than the corresponding industrial sample. These sites must receive industrial discharges that we were not aware of. The Sorel site is located downstream from the confluence of the St. Lawrence and Richelieu rivers. The Richelieu is a large tributary ($\approx 350 \text{ m}^3 \text{ sec}^{-1}$) that discharges sanitary and agricultural wastes into the St. Lawrence at Sorel (MENVIQ, 1993). Satellite images confirm that the sampling site at Sorel is receiving Richelieu river discharge (Létourneau, pers. comm.). The other two outliers (Boucherville, La Prairie) likely receive sanitary wastes that we did not examine in our previous investigations. In 1991, several municipalities located on the south shore of the St. Lawrence did not have municipal wastewater treatment facilities.

The results presented in Figure 8 and models 6 and 7 provide empirical evidence that bottom sediments are contaminated with substances of industrial origin. Regions that experience higher loadings of particle bound genotoxins also contain sediments that are more genotoxic. Similar to the results presented in Figure 7, the results presented in Figure 8 indicate that Boucherville receives genotoxins from additional, unknown sources. The maximum loading of dissolved base/neutral genotoxins is also related to the potency of downstream bottom sediments. White et al. (1995a) determined that the concentration of suspended solids in industrial effluents has a positive effect on the concentration of dissolved genotoxins. At very high concentrations of suspended solids (10^2 - 10^3 mg per L), increasing amounts of base/neutral genotoxins were found in the aqueous filtrate (White et al., 1995a Figure 2). The relationship identified in model 7 is likely a consequence of a post-emission drop in the concentration of suspended solids. The drop results in an increased sorption partition coefficient and a concomitant increase in the concentration of genotoxins adsorbed to suspended particulate matter. A general discussion on the effect of sorbent concentration on solute partitioning is available in O'Connor and Connolly (1980).

Comparisons of the direct acting SPM samples with the S9 activated BSED samples presents an interesting dilemma. Since the bottom sediments must contain particles removed from the water column, it is not immediately apparent how sedimented particulate matter can have little or no direct acting substances and high levels of S9 activated substances. Part of the problem lies in confirming the presence of pro-genotoxic substances in the water column. If decreases in activity are due to the physical effects of S9 proteins, it may be argued that responses measured in the presence of S9 cannot be interpreted. However, the BSED results (corrected for sand content) indicate that the potencies of some samples with S9 are greater than the potency of the corresponding SPM sample without S9 (e.g. Montreal-East). If the SPM sample contained similar levels of progenotoxic substances, the addition of S9 should have produced an increase in potency. The principles of mass balance suggest that there are two ways by which this can occur. BSED samples that have high levels of progenotoxins (e.g. Montreal-East, Jonquière) do not reflect current levels of genotoxins in the water column. While this may be partially true, the fact remains that none of the SPM samples produced SRIP values that are higher with S9. Alternatively, the results suggest that direct-acting substances sorbed to particulate matter in the water column are being converted into more stable pro-genotoxic substances in the bottom sediments. Rather than the aforementioned rapid decay of direct-acting substances, direct-acting substances *removed* from the water column may be appearing in bottom sediments as pro-genotoxic substances. At the present time we cannot present any mechanistic evidence for this conversion from direct acting to progenotoxic. However, there is evidence in the literature to support the rapid interaction of direct acting substances in surface waters with nucleophiles such as sulfite (Cheh et al., 1980). In addition, the results presented in Figure 8a confirm an empirical relationship between the S9 activated genotoxicity of bottom sediments and maximum loading of particle bound genotoxins, a value primarily determined by direct acting genotoxicity (see White et al., 1995b).

As a corollary to the above discussion, the actual rate at which genotoxins are degraded and completely removed from the system can only be determined via a comparison of the total genotoxicity of SPMs with the total genotoxicity of BSEDs, where total is defined as the sum of direct acting and S9 activated only when S9 activation leads to an increase in the observed response. The calculation of total genotoxicity in this manner is supported by the aforementioned results of Courtois et al. (1988 and 1992). The results summarized in Table 7 indicate that for three sites (Montreal-East, Jonquière and St. Romuald) the total genotoxic potency of the older sedimented material is greater than that of the fresh material collected in the water column. Since bottom sediments can provide an historical record of contaminant accumulation (Wetzel and Likens, 1991; Carignan et al.,

1994; Smith and Levy, 1990; Gearing et al., 1992; Durham and Oliver, 1989), this result must reflect declining emission rates. Several documents confirm that contaminant loadings at Jonquière and Montreal-East have decreased over the past 20 years. Smith and Levy (1990) indicate that release of PAHs by aluminum refineries in the Jonquière region decreased in the mid to late 1970's. Financial difficulties and increasingly strict government regulations forced dramatic reductions (>60%) in the production of refined petroleum products at Montréal-East (CSL, 1992; Birmingham, pers. comm.). Comparisons of total BSED SRIP values and total SPM SRIP values for Boucherville, Donnacona and Trois Rivières can be used to calculate the actual rate at which genotoxins are lost from the system. Assuming exponential decay, and sedimentation rates of $1-2 \text{ mm yr}^{-1}$, the loss rate would be $\approx 1.5-24 \% \text{ yr}^{-1}$ (half-life $\approx 3-45$ years). More accurate determination of loss rates will require more detailed knowledge of sedimentation in these areas. Unfortunately, the areas discussed are transitional zones that experience variable sedimentation (Carignan, pers. com.; Pelletier, pers. comm.).

Despite a complete lack of direct industrial input, the sample collected at Lake Memphremagog was found to be highly genotoxic (see Table 4). The high potency per unit dry solid is partially due to the low concentration of suspended particulates. However, even if the SPM results are expressed per liter of surface water they still exceed most of the St. Lawrence and Saguenay river values. In the absence of industrial activity, the genotoxins are likely produced and emitted during recreational activities. The lake receives a large number of pleasure boats that contribute combustion by-products, fuel oils and sanitary wastes to the lakewater. Yumlu (1994) determined that the average pleasure boat discharges as much as 60 mg of particulate matter and 1 g of hydrocarbons per day. In addition, there is a dramatic difference between the suspended particulates in the two systems. Particles carried in the St. Lawrence river are primarily inorganic, with organic matter contents rarely exceeding 20% (LOI as % dry wt, NAQUADAT, 1992). In contrast, particulate matter from L. Memphremagog is composed of planktonic biota (LOI $\approx 80\%$ dry wt.) that are capable of actively and passively concentrating organic contaminants in the water column. Furthermore, several researchers have documented the ability of both plants and algae to activate pro-genotoxic substances such as those in combustion by-products (Harwood et al., 1989; Wagner et al., 1990).

In our previous research we examined the potency, partitioning and potential hazard of genotoxins in industrial effluents. This study extended those analyses and examined genotoxins adsorbed to suspended and sedimented particulate matter in the downstream ecosystem. Figure 9 provides a summary of the results obtained and illustrates possible post-emission pathways of industrial genotoxins. The ultimate fate and effects of sorbed

genotoxins will be determined by a complex series of mechanical, chemical and biological processes that control particle movement and chemical-particle interactions (Knezovich et al., 1987). Effects on indigenous biota will be primarily determined by bioavailability. Uptake and effects of particle-bound substances on pelagic biota can proceed via ingestion and direct desorption from particulates (Fox et al. (1983), passive transfer from particulates to planktonic organisms (Harding and Phillips, 1978) or consumption of contaminated prey (Borgman and Whittle, 1983; Rasmussen et al., 1990). While similar processes occur in bottom sediments, contamination of benthic biota will also be effected by physical and biological processes that determine sediment resuspension, redistribution (Karickhoff and Morris, 1985; Elder et al., 1979) as well as contaminant desorption (O'Connor and Connolly, 1980) and degradation (Swartz and Lee, 1980).

Figure 9. Summary of the results obtained in this study. Particle-bound genotoxins released into the receiving system are initially diluted with entrained *fresh* water. Diluted suspended particulate matter is approximately an order of magnitude less potent than that found in industrial effluents. Following dilution, sorbed genotoxins can be removed from the water column by degradation, transportation and sedimentation. The results obtained suggest that once sedimented, direct acting substances, which predominate in the water column, are transformed into progenotoxic substances that require S9 activation. Progenotoxic substances in bottom sediments can accumulate or be lost through burial and further degradation. Comparisons of SPM and BSED total potency values indicates that annual losses are several percent. Annual or seasonal fluctuations in current may cause erosion and resuspension of particle-bound genotoxins. Dashed arrows indicate pathways that were not investigated.

Fate of Industrial Genotoxins in the Downstream Environment

Industrial Effluent- Suspended Particulates
 0.8 - 1500 µg BaP Equiv. per g dry weight
 Geometric mean = 78 µg BaP Equiv. per g
 S9 activated rarely > direct acting

Discharge
 0.4 - 31000 µg BaP Equiv. per day.
 $K_d \approx 10^2 - 10^7$

Dilution
 Entrainment of river water
 into effluent discharge(s)
 Whole water = 0.35 ng BaP
 Equiv. per g (Results from
 Langevin et al., 1992;
 1 g = 1 mL)

Water Column- Suspended particulates
 0.5 - 50 µg BaP Equiv. per g dry wt.
 Geometric mean = 6 µg BaP Equiv per g
 S9 activated never > direct acting

Transportation

Transformation
Degradation
Sedimentation

Erosion
Resuspension

Accumulation
Degradation
Burial

Bottom sediments-
 No S9: rarely positive (0.6 - 11 µg BaP Equiv. per g)
 With S9: Always > than No S9
 0.2 - 113 µg BaP Equiv per g dry wt.
 Geometric mean = 4 µg BaP Equiv per g

ACKNOWLEDGEMENTS

We are grateful to several people for their support, assistance and advice. Richard Carignan of the, University of Montreal, Emilien Pelltier of the Department of Fisheries and Oceans (Mont-Joli, Quebec) and Stéphane Lorrain of Environment Canada (Montreal) for providing information about sedimentation rates in the St. Lawrence and Saguenay rivers. Jay Leopkey, Brigid Payne, Cornelia den Heyer and Brian Walker for skilful technical assistance in the field and the laboratory. The study was supported by a St. Lawrence Center-Natural Sciences and Engineering Research Council of Canada research partnership grant to JBR and a Natural Sciences and Engineering Research Council of Canada doctoral research fellowship to PAW.

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CHAPTER 6

THE GENOTOXIC HAZARD OF DOMESTIC WASTES IN SURFACE WATERS

INTRODUCTORY REMARKS

The surface water genotoxicity results published by Langevin et al. (1992) indicated that samples collected in the Montréal area are more contaminated than those collected upstream. This chapter presents the results of a mass balance analysis that investigated whether the industrial and municipal wastewater sources studied in chapters 3 and 4 can account for this local increase in genotoxicity. The results presented are a synthesis of data from chapters 3, 4 and 5, in addition to data published by Langevin et al. (1992).

The material presented has been prepared for submission to *Nature*. As such, it has been prepared according to guidelines for publication in *Nature*. For example, the main body of the text is not subdivided into the traditional sections employed in more specialized scientific journals. Methodological details are contained in the lengthy figure legends. In addition, numbers are used as citation markers. Prof. Joseph Rasmussen played a role in the development of the ideas presented. He is the second author of the manuscript.

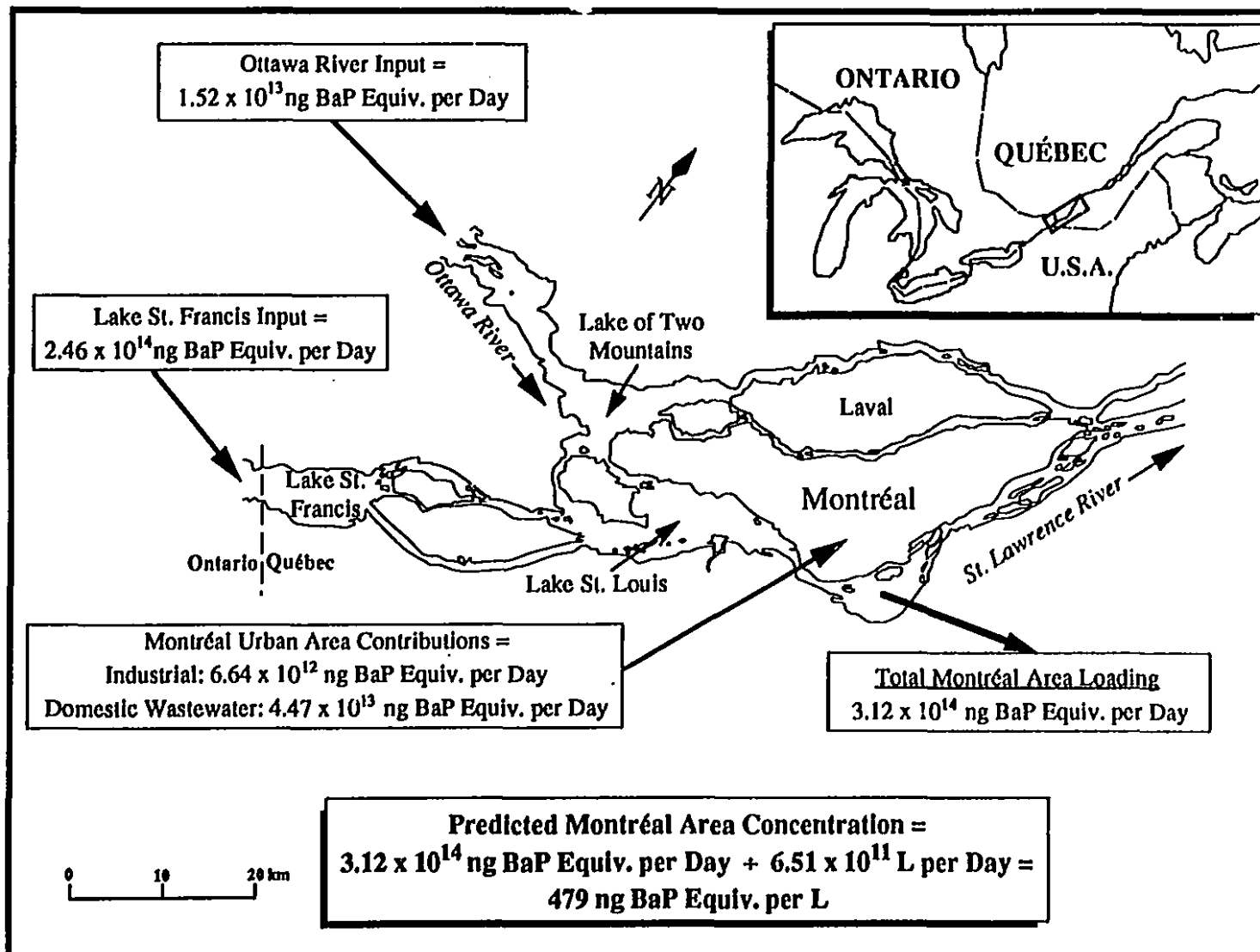
Appendix 8 contains the data used to generate the models, figures and tables.

Domestic wastewaters are consistently genotoxic and characterized by high daily discharge. Mass balance analyses based on SOS Chromotest results indicate that most of the genotoxic activity in surface waters can be accounted for by domestic rather than industrial inputs. Surface water levels along the St. Lawrence-Great Lakes system can be predicted solely from demographic and hydrological information. Ames/*Salmonella* test mutagenicity levels in other major rivers throughout the world show similar patterns in relation to demography and hydrology. While the putative genotoxins and mutagens in surface waters remain to be identified, previously identified fecal mutagens are likely contributors.

Rivers, lakes and harbors are frequently the recipients of large amounts of genotoxic industrial and municipal wastes (1-5). Environmental monitoring efforts have been directed primarily at industrial inputs because of the high potency (effect per unit mass or volume) of these wastes (1, 2, 4, 6-7). However, assessment of potential hazard requires knowledge of discharge in addition to potency (7-8). Domestic wastewaters, although ranking low in genotoxic potency (1, 2, 5, 9, 10), generally exceed industrial discharge rates by an order of magnitude or more (1, 11-14); however, their genotoxic contribution has received much less attention. Several components of domestic waste have been shown to be genotoxic. These include human feces and urine which can contain potent genotoxins (15-18) including the fecapentaenes (19-20), in addition to combustion byproducts (21-22) that enter the municipal system via street runoff (23-24). In this study we present a mass balance analysis that determines the relative contributions of domestic and industrial waste streams to surface water genotoxicity of the St. Lawrence River around Montreal, the results of which were used to model genotoxins throughout the Great Lakes-St. Lawrence system.

In an earlier study (25) we used the SOS Chromotest (26) to measure the genotoxicity of St. Lawrence river surface water and observed that Montreal area samples are more potent than those collected upstream. Here we quantify the loadings (product of potency and discharge) from domestic and industrial sources and show that these can account for the increased genotoxicity of Montreal area waters. Since genotoxins in surface- and wastewaters are chemically diverse, the mass balance calculations required us to express toxicity as equivalents of a standard reference compound. All genotoxicity values were thus converted to their BaP (benzo(a)pyrene) genotoxic equivalence and expressed as ng BaP per L. The results indicate that 87% of the Montreal area genotoxic contributions to St. Lawrence surface waters can be accounted for by domestic wastewaters (Figure 1), with

Figure 1. Mass balance of genotoxicity for surface waters of the St. Lawrence river at Montreal. Inputs to the Montreal area were divided into three categories- those arriving from the Ottawa River in the west, those arriving from Lake St. Francis in the southwest and local discharges from the Montreal metropolitan area. Ottawa River and Lake St. Francis loading values were calculated as the product of the geometric mean surface water genotoxic potency (25) and the mean river discharge (27-28). Industrial and municipal wastewater contributions from the Montreal region were determined using our effluent results (1) and data supplied by the City of Montreal (29). The 12 largest industries in the Montreal region, the MUC (Montreal Urban Community) wastewater treatment facility and the regional inhabitants not connected to the MUC wastewater treatment facility (see below) emit a total of 51.3 kg of BaP equivalents day⁻¹. 8.8% of this quantity is accounted for by known inputs from large paper manufacturing, metal refining and founding, metal surface treatment, petroleum refining and organic chemical production facilities (1). An additional 4.1% of the total, regional loading is accounted for by small, unknown industrial discharges (see below). The remaining 87.1% of the regional genotoxic load can be accounted for by domestic emissions. The predicted surface water concentration of 479 ng BaP equivalents L⁻¹ is close to the geometric mean of Montreal area surface water measurements, 510 ng BaP equivalents L⁻¹ (25). The population serviced by the MUC municipal wastewater treatment facility (1.4 million) and the domestic loading (28.4 kg day⁻¹) were used to calculate a daily, *per capita* genotoxic emission rate of 20.3 mg BaP equivalents person⁻¹ day⁻¹. This value was used to account for domestic loadings from 0.8 million inhabitants of the drainage basin that are not connected to the MUC domestic wastewater collection system. The proportion of the MUC wastewater loading from unknown small industries was calculated using the total daily discharge of unknown small industries (14.8% of total MUC discharge by volume (29)) and the geometric mean genotoxic potency of 50 industrial effluents obtained from the most hazardous industries discharging waste into the St. Lawrence river (1). The results indicate that 6.8% of the total MUC load can be attributed to unknown small industries. An additional 2.0% can be attributed to the effluents of 3 large industries connected to the MUC collection system (1). Conversion of genotoxicity measurements (25, 1) to their benzo(a)pyrene (BaP) equivalence employed the mean SOS Chromotest MDGC value for benzo(a)pyrene. 0.0757 µg assay mL⁻¹ is based on 40 measurements made in our laboratory. Discharge values in L day⁻¹ are means of monthly measurements taken between June and September, 1988 (25, 27, 28). Ottawa river inputs were adjusted to account for discharge that does not immediately enter the St. Lawrence system. Genotoxic loading and potency values are all maximum values calculated under activation conditions that produced the strongest response.



domestic indicating wastes that are not of industrial origin. Although municipal wastewaters can contain industrial emissions that contribute to the final genotoxic potency of the waste (5, 9, 30), the proportion of the total daily discharge that is of industrial origin is rarely more than 20% (9, 12, 14, 31).

The upstream input from Lake St. Francis accounts for almost 80% of the genotoxicity observed in Montreal area surface waters. Thus, the concentration of genotoxins in upstream inputs should reflect the number of inhabitants in the drainage basins of the upstream Great Lakes. We therefore collected demographic and hydrological information about the Great Lakes and their associated drainage basins to determine if upstream domestic wastewater emissions alone can account for the genotoxicity of waters entering the Montreal area. The results obtained are summarized in Table 1. The predicted surface water concentration in Lake St. Francis is 224 ng BaP Equiv. per L, the observed value is 409 ng per L. While the $\approx 45\%$ discrepancy between the observed and predicted Lake St. Francis values can likely be accounted for by the considerable quantity of industrial emissions east of the St. Clair River (32), it is likely within the error of our mass balance calculations. This result, in combination with the fact that 87% of the Montreal area contribution to regional surface water genotoxicity is accounted for by domestic wastewater emissions, provides further support for domestic wastewater as the major source of genotoxins in surface water.

We extended our analyses to determine if the levels of genotoxins in other rivers can be predicted solely from demographic and hydrological information. We collected published surface water mutagenicity data for four European rivers (36-42), one Japanese river (43), and one South African river (44). Since the SOS Chromotest has not been used in many examinations of surface water, we collected data from studies that used the Salmonella/Microsome assay or *Ames* test (45). The number of inhabitants occupying the drainage basins of these rivers above the point of sample collection ranged from 1 to 31 million (46-47, or most recent census). The mass balance results for the St. Lawrence-Great Lakes system, presented in Figure 1 and Table 1, indicate that there should be a close, linear relationship between surface water mutagenicity and variables that reflect the regional loading of domestic wastes. The results obtained indicate that there is an excellent, linear relationship between surface water mutagenicity and population to discharge ratio (Figure 2). Furthermore, when we use the mean potency of benzo(a)pyrene on *S. typhimurium* strain TA98 (701.9 revertents μg^{-1} mL top agar⁻¹, 53-57) to express mutagenicity results as their BaP equivalent and calculate a *per capita* daily genotoxic output value we obtain 14 mg BaP equivalents day⁻¹. This value is close to that obtained from our SOS Chromotest analyses of domestic emissions from the city of Montreal (see Figure 1

Table 1. Total genotoxic output of each Laurentian Great Lake.

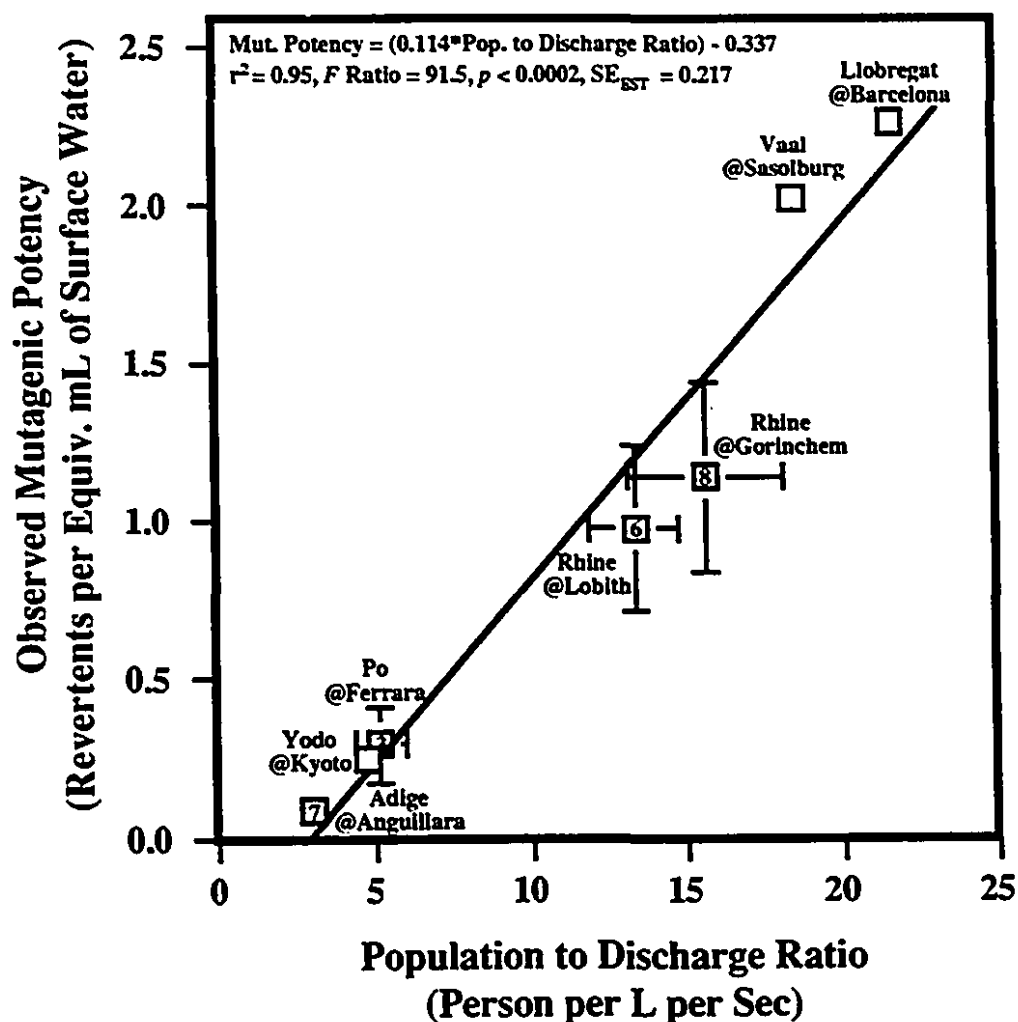
Lake	Drainage Basin Population	Loss to Decay (%)	Loss to Sedimentation (%)	Total Genotoxic Output† (ng BaP Equiv. per Day)
Superior	0.74×10^6	89.9	6.4	5.47×10^{11}
Michigan	13.97×10^6	82.4	11.2	1.83×10^{13}
Huron	2.37×10^6	58.4	21.1	1.38×10^{13}
Erie	12.97×10^6	17.0	32.5	1.40×10^{14}
Ontario	6.64×10^6	37.5	14.0	1.33×10^{14}
Additional St. Lawrence river contributions*	0.42×10^6	0.0†	0.0†	8.53×10^{12}
Total Loading at Cornwall, Ontario				1.42×10^{14}

*St. Lawrence river between the outflow of Lake Ontario at Kingston, and the start of Lake St. Francis at Cornwall.

†Current speed between Kingston and Cornwall is 0.5 to 4 knots. The travel time of <5 days is too short for significant decay or sedimentation.

‡The daily output of each lake was calculated from demographic data (32), a *per capita* genotoxic emission rate (see Fig. 1 legend) and hydrological information (32). Unlike the St. Lawrence river near Montreal, genotoxins exported from the Great Lakes can be lost to sedimentation and decay. Sedimentation losses were calculated using the sorption partition coefficient of genotoxins in domestic wastewaters (33), surface water concentrations of particulate matter (34) and particle settling rates calculated from the mass balance parameters of Rowan and Rasmussen (34). Decay losses were calculated using a decay coefficient of 0.13 yr^{-1} (35). The total loading at Cornwall is the sum of the genotoxic output from Lake Ontario and the contributions of municipalities located along the St. Lawrence river between the outlet of Lake Ontario at Kingston and the start of Lake St. Francis at Cornwall. The ratio of the total genotoxic loading received at Cornwall ($1.42 \times 10^{14} \text{ ng BaP equiv. day}^{-1}$) to the mean discharge of the St. Lawrence river at Cornwall ($6.34 \times 10^{11} \text{ L day}^{-1}$ (28)) provides the predicted surface water concentration of genotoxins in Lake St. Francis- $224 \text{ ng BaP equivalents L}^{-1}$. The observed geometric mean surface water genotoxicity value is $409 \text{ ng BaP equivalents L}^{-1}$ (25). U.S. population data are 1980 values. Canadian data are 1981 values. Calculation details can be found in Appendix 10.

Figure 2. The relationship between (direct-acting) surface water mutagenicity and population to discharge ratio for 6 rivers throughout the world. Mutagenic potency values, corrected for filtration losses, were converted to his revertents per equivalent mL of whole water. Mutagenicity of surface water extracts was measured on *Salmonella typhimurium* TA98. Although other strains of *Salmonella* have been used to detect surface water mutagens, several researchers have noted that surface water mutagens are predominantly frameshift mutagens (36, 37, 44, 48). Corrections for filtration losses are based on the mean sorption partition coefficient for genotoxins present in domestic wastewaters (32) and the surface water concentration of suspended particulate matter (49, 50). Where the concentration of suspended particulate matter was not available, 25 mg per L was used. All potency values were standardized to a top agar volume of 1 mL. Where the top agar volume was not provided, volumes of 2.2 mL (no S9) and 2.7 mL (+S9) were assumed (51). Population data are from Prat et al. (46), CIPR (47) or the most recent census available. Discharge data are from van der Leeden (52) and Navazio (53). Wherever possible discharge values reflect the season and year of sample collection. Where several observations were available, values presented are arithmetic means. Where error bars are shown, they represent one standard error of the mean. Error bars for Adige river values are too small to be shown. Number of observations is indicated on the relevant plotting symbols. The linear relationship indicated was determined using ordinary, least-squares linear regression. In all cases, time delays between emission and sample collection were deemed too short to permit significant decay or sedimentation. For example, Rhine river water travels from Lake Constance to the German-Dutch border in less than 10 days (49). Analyses based on S9-activated measurements revealed a similar relationship. Linear regression analysis of mutagenic potency (+S9) against population to discharge ratio provided an r^2 of 0.88 and slope of 0.195.



legend). This reinforces our conclusions about the role of domestic wastewater in determining the levels of genotoxins in surface waters and underscores the comparability of the two bioassays and the efficacy of the equivalence approach.

In a mass balance study using our surface water genotoxicity data (25) and wastewater loading measurements (1), we have demonstrated the importance of domestic wastewater emissions in determining the concentration of genotoxins in surface waters of the St. Lawrence-Great Lakes system. Further confirmation was accomplished using published surface water mutagenicity data for 6 rivers throughout the world. These results indicate that surface water mutagenicity levels can be predicted solely from demographic and hydrologic data. Overall, the results obtained suggest that a more detailed breakdown of the genotoxic contributors to the domestic waste stream is required. Furthermore, it is apparent that genotoxicity is not removed, and may even be exacerbated by, conventional wastewater treatment processes such as chemical coagulation, sedimentation and disinfection (3, 9, 10, 30, 58-59). Determination of the genotoxins in raw and treated domestic wastewaters that are responsible for observed surface water levels seems a promising area for further research.

ACKNOWLEDGEMENTS

We gratefully acknowledge the assistance of several people. Diedrich Backhaus of the Landesanstalt für Umweltschutz Baden-Württemberg for providing Rhine river information. Paola Venier of the University of Padova for providing Adige and Po river information. Fernand Cadieux, Pierre Purenne and Michael Davidson of the Service de l'environnement, Communauté Urbaine de Montréal for providing essential information about the MUC wastewater collection and treatment system. John Meier of USEPA-Cincinnati, Stanislas Pauwels of Exxon Biomedical Sciences, Virginia Houk of USEPA-Research Triangle Park, Saburo Matsui of Kyoto University, Kay Ho of USEPA-Naragansett, Chantale Côté of Beak Consultants Ltd., as well as Neil Price and Marc Trudel of McGill University for thoughtful comments. Strahan Tucker for compiling German, Dutch, French, Belgian, Swiss, Austrian, Italian and South African census data. This project was funded by a Natural Sciences and Engineering Research Council of Canada operating grant to JBR and the Vineberg Family Award from McGill University to PAW.

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CHAPTER 7

DETECTION OF GENOTOXIC SUBSTANCES IN BIVALVE MOLLUSCS FROM THE SAGUENAY FJORD (CANADA), USING THE SOS CHROMOTEST

INTRODUCTORY REMARKS

The next logical step in the research project is the investigation of indigenous biota that inhabit regions which receive genotoxic industrial discharges. The first step in these investigations is the demonstration that the SOS Chromotest can be used to detect genotoxicity in tissue extracts. This chapter describes the use of the SOS Chromotest to investigate the accumulation of SOS genotoxins in *Mya arenaria* from the Saguenay Fjord. Regions investigated are located downstream from several of the industries examined in chapters 3 and 4.

The material presented was prepared for submission to *Environmental Toxicology and Chemistry*. Both Prof. Joseph Rasmussen and Dr. Christian Blaise played a role in the development of the ideas presented. Dr. Blaise's experience with *Mya arenaria* resulted in a substantial contribution to the final manuscript. As such, he is second author. Prof. Rasmussen is third author.

Appendix 9 contains the data used to generate all figures and tables.

ABSTRACT

Many researchers have used chemical analyses to document the accumulation of genotoxic and carcinogenic substances (e.g. polycyclic aromatic hydrocarbons) in bivalve molluscs. Although bioassays have some distinct advantages over chemical analyses, few studies have employed them to investigate the accumulation of genotoxins in aquatic biota that inhabit areas contaminated with industrial and municipal wastes. This study employed the SOS Chromotest, a short-term bacterial genotoxicity assay, to investigate the presence of genotoxins in bivalve molluscs from the Saguenay Fjord (Canada). Genotoxicity analyses were performed on dichloromethane extracts of *Mya arenaria* and *Mytilus edulis* collected downstream from several aluminum refineries and forestry products industries. The results confirmed that bivalve molluscs inhabiting regions downstream from industries known to produce and release genotoxic substances are contaminated with both genotoxins and polycyclic aromatic hydrocarbons. The results did not reveal a clear downstream trend of decreasing genotoxicity with increasing distance from the presumed industrial sources(s). Relationships between demographic variables (e.g. population near shoreline) and genotoxic potency suggest that bivalves are accumulating genotoxic substances of domestic origin. A significant effect of drainage basin area on tissue PAH levels indicates that bivalves are accumulating PAH emissions transported in the atmosphere. In spite of contamination with known progenotoxic PAHs, the addition of rat liver microsomal enzymes reduced the genotoxic potency of all samples investigated. Although the origin and identity of genotoxins in bivalves of the Saguenay Fjord remains to be determined, the study has confirmed the utility of the SOS Chromotest in environmental monitoring of aquatic biota.

INTRODUCTION

The direct discharge of genotoxic industrial wastes into lakes, rivers, harbors and streams is well documented (see review by Houk, 1992). However, most research efforts have only measured the genotoxic or mutagenic potency of these wastes, and failed to quantify the potential hazard posed to the downstream environment and its associated biota. In an effort to quantify potential genotoxic hazard, White et al. (1995a) used daily discharge and SOS Chromotest genotoxic potency data to calculate the daily genotoxic loadings of a wide range of industrial and municipal wastewaters. While their results documented the loadings from 42 wastewater sources, they did not investigate exposure of downstream biota or concomitant genotoxic effects. Despite a lack of understanding of the post-emission behaviour of industrial genotoxins, many researchers have used short-term bacterial assays to document accumulation in sediments (e.g. Ho and Quinn, 1993; Balch et al., 1995; Marvin et al., 1993; Durant et al., 1992) as well as some aquatic biota (e.g. Marvin et al., 1994; Parry et al., 1980; Rodriguez-Ariza et al., 1992). In addition, genotoxic and carcinogenic effects have been observed in biota that inhabit areas known to be contaminated with industrial wastes (e.g. Malins et al., 1984; Nacci et al., 1992; Mix, 1986; van der Oost et al., 1994; Gardner et al., 1991a; Gardner et al., 1991b; diGiulio et al., 1993; Mix, 1988).

Most efforts to monitor the aquatic environment for mutagens have employed the *Salmonella*/mammalian microsome assay or Ames test (Ames et al., 1973; Ames et al., 1975). However, histidine contamination of tissue extracts and other biological samples hampers the performance of the test and complicates the quantification of prototrophic reversions attributable to mutagens in the sample (Sparks et al., 1981; Parry and Al-Mossawi, 1979; Marvin et al., 1994). As a result, relatively few studies have investigated the accumulation of genotoxic substances in aquatic biota. Since the advent of the Ames test, several alternative short-term genotoxicity assays have been developed. The SOS Chromotest, first described by Quillardet et al. (1982), uses the error-prone DNA repair pathway of *Escherichia coli* for the detection of DNA damaging agents. Since its introduction, several studies (Ohta et al., 1984; Quillardet et al., 1985; Quillardet and Hofnung, 1993; Mersch-Sundermann et al., 1994; White and Rasmussen, 1995a) have demonstrated both qualitative and quantitative correspondence between the SOS Chromotest and the more traditional Ames test. Since the SOS Chromotest does not rely on prototrophic reversion, it can easily accept biological samples such as urine and faeces (Fish et al., 1987; Kohn et al., 1988; De M  o et al., 1988). Although the test system has been used for the detection of genotoxins in surface waters (Langevin et al., 1992; Wong et

al., 1994), sediments (Lan et al., 1991; Xu et al., 1987; Langevin et al., 1992; White et al., 1995b) and soils (McDaniels et al., 1994), it has never been used to investigate the accumulation of genotoxins by aquatic biota inhabiting contaminated regions.

The Saguenay-Lake St. Jean region of Quebec is well known for its high concentration of industries which have contributed to the contamination of the Saguenay Fjord, a major tributary of the St. Lawrence River (Cossa, 1990; Steel, 1995). In addition to mercury pollution from the chlor-alkali industry, the region is well known for PAH (polycyclic aromatic hydrocarbon) contamination from the aluminum industry (Gearing et al., 1994; Smith and Levy, 1990; Martel et al., 1987; Steel, 1995; Cossa, 1990). Ready accessibility to the sea and ample hydroelectric resources led to the construction of several large aluminum smelters between 1926 and 1945 (ALCAN, 1970). By 1960 the region had become one of the world's largest producers of aluminum (Smith and Levy, 1990). Between 1938 and 1980 over 45 000 tons of PAHs (≈ 5 kg PAH per ton Al) were emitted by Al smelters in the region (Smith and Levy, 1990). Both genotoxic and carcinogenic PAHs of pyrolytic origin are known to be present in these emissions (IARC, 1984). Genotoxic and carcinogenic PAHs of pyrolytic origin have been found in sediments of the Saguenay Fjord (Martel et al., 1986; Martel et al., 1987; Smith and Levy, 1990). In addition, several researchers have documented an accumulation of unsubstituted PAHs in the common blue mussel *Mytilus edulis* (Cossa et al., 1983; Picard-Berubé et al., 1983), an organism renowned for its limited ability to metabolize and excrete PAHs (McElroy et al., 1989; James, 1989).

This study employed a semi-automated version of the SOS Chromotest to investigate the presence of genotoxins in aquatic biota inhabiting the Saguenay Fjord. Since bivalve molluscs are sedentary, relatively long-lived suspension feeders known to accumulate genotoxic PAHs such as benzo(a)pyrene (Andelman and Suess, 1970; Dunn and Stich, 1975; Mix and Schaffer, 1979), examinations of biota focussed primarily on the soft-shell clam (*Mya arenaria*), and to a lesser extent on the common mussel (*Mytilus edulis*).

MATERIALS AND METHODS

Study Site and Sample Collection

Samples were collected in the province of Quebec in an area known as the Saguenay-Lake St-Jean Region (Figure 1). The region is characterized by the Saguenay River located between the outflow of Lake St-Jean and the start of the Saguenay Fjord approximately 15 km east of the town of Chicoutimi. The Saguenay Fjord is a long, narrow and deep (maximum depth $\approx$ 275 m) basin that extends approximately 170 km from the mouth of the Saguenay river to the St. Lawrence river estuary at Tadoussac. The region between Alma and La Baie contains several large forestry products and metal processing industries. Table 1 provides a list and description of the major industries in the region. Biota samples were collected along the Fjord and in the St. Lawrence estuary immediately adjacent to the Fjord during October 1992. The locations of all sampling sites are indicated on Figure 1. Samples were collected from the intertidal zone at low tide. Only whole undamaged organisms were collected. Samples were placed in plastic bags and immediately frozen on dry ice. To investigate intra-site variability three replicate samples, designated east, west and center, were taken at Anse St-Étienne.

Preparation of Tissue Extracts

Tissues were separated from shells and placed in a large preweighed beaker. Excess water was decanted and the fresh weight measured. Where sample size permitted, samples were divided into two sub-samples. Composite (50 g) tissue samples were extracted using a combined blender/sonication method. The method, described by White et al. (1995a), is a variation on those developed by Marble and Delfino (1988), Maggard et al. (1987) and Williams (1989). Briefly, tissue samples were blended with 400-500mL of dichloromethane (Anachemia Science, Montreal) in stainless steel cups using a high-speed (3500 rpm) industrial blender (Eberbach Corp., Ann Arbor, MI). Each sample was blended for 3 x 4 minutes and subsequently sonicated (ultrasonic cell disrupter- Branson Sonic Power Co., Danbury, CT) on ice for 2 x 3 minutes. Blending and sonication periods were alternated with 3-4 minute cooling periods. The method was validated by spiking a sample of freshwater clams (Unionidae) with a mixture of 15 polycyclic aromatic hydrocarbons (Supelco PAH Mix 4-8905, Supelco Canada Inc., Oakville, Ontario). Mean recoveries of 3-, 4- and 5-ring PAHs were 53%, 85% and 86% respectively. A matrix blank, mixed freshwater invertebrates from a site that receives no direct industrial input, was analyzed in the same manner as all other samples.

Figure 1. Study area and sampling sites. Seven of the nine sampling sites are located in the Saguenay Fjord between La Baie des Ha! Ha! and Tadoussac. Two sites, Baie Ste-Catherine and Baie du Moulin à Baude, are located just outside the Fjord in the St. Lawrence estuary. Samples of soft-shell clams (*Mya arenaria*) were collected from each site except Baie Ste-Catherine where only the blue mussel (*Mytilus edulis*) was available. In addition to *Mya arenaria*, *Mytilus edulis* was also collected at Baie du Moulin à Baude and Anse à la Barque. DBA = drainage basin area in km², POP = drainage basin population. Drainage basin areas were measured on 1:50 000 topographic maps. Population values are from the 1991 census of Canada (Statistics Canada, 1993). For drainage basins that do not contain any towns or villages indexed in the census, the number of private dwellings (from topographic maps) was multiplied by the mean number of persons per dwelling in that region (Statistics Canada, 1993).

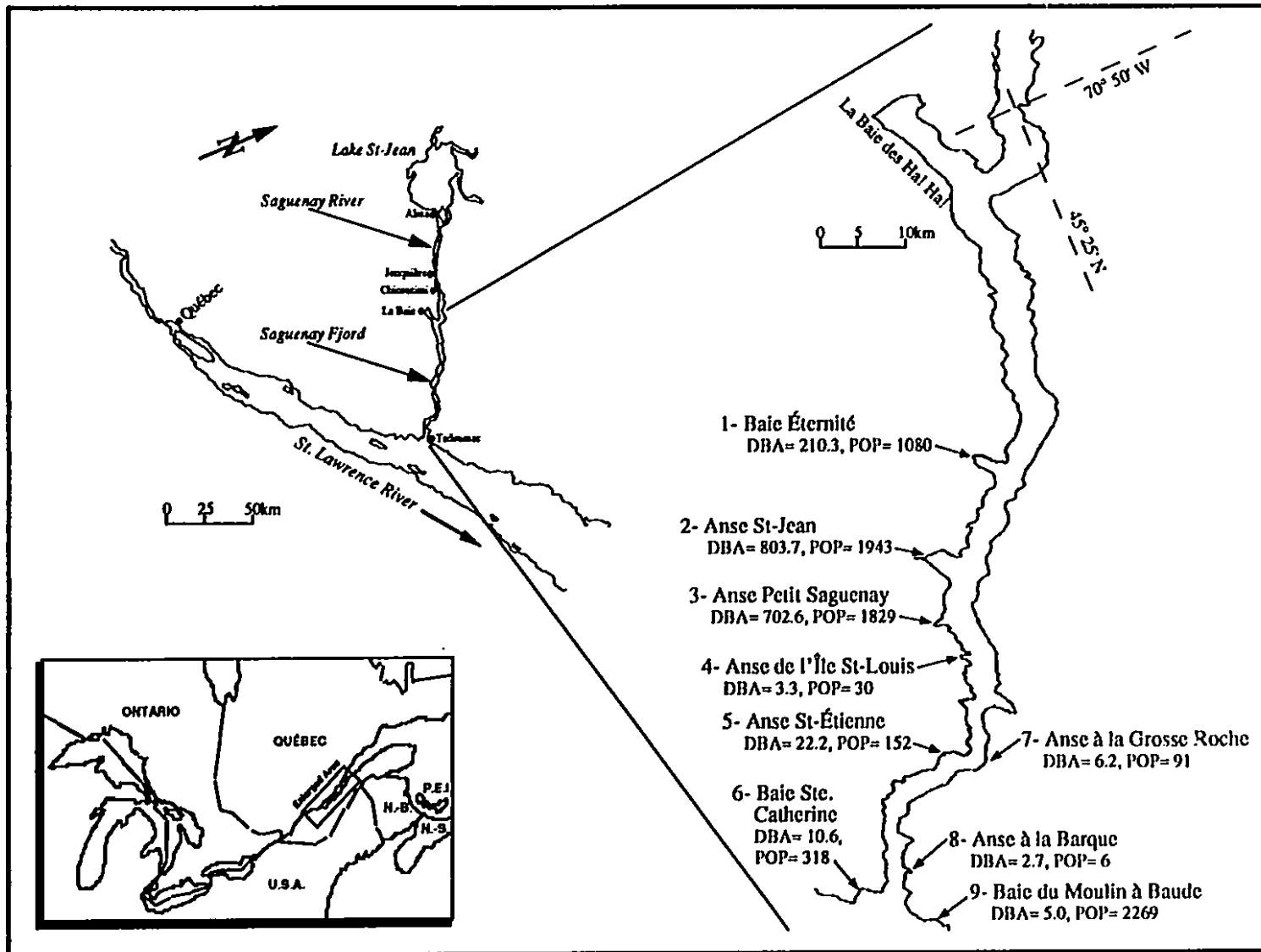


Table 1. Description of major industries discharging wastes directly into the Saguenay river and Saguenay Fjord between Alma and La Baie. Principal pollutants released are based on chemical analyses conducted by Environment Canada under *The St. Lawrence Action Plan*. Daily discharge values are averages based on 2-5 day monitoring periods. Genotoxic loading values are based on SOS Chromotest analyses of dichloromethane extracts from final effluents (White et al, 1995a). Values were converted to grams B(a)P (benzo(a)pyrene) genotoxic equivalents using the mean SOS genotoxic potency of B(a)P. NT- indicates that final effluent samples were subjected to chemical analyses, but not directly tested for SOS genotoxicity. Plus symbols indicate total daily loadings of identified genotoxins: + indicates > 1 g genotoxic material day⁻¹, +++ indicates > 1 kg genotoxic material day⁻¹. Discharged genotoxins include a variety of PAHs, resin acids and chlorinated guaiacols.

Industry	Product/Process	Location	Major Pollutants	Mean Daily Discharge (L day ⁻¹)	Genotoxic Loading (g B(a)P equiv. day ⁻¹)	Reference*
Alcan Ltd. (Île-Maligne facility)	Al ingots/Soderberg process	Alma	F, Al, PAHs	1.3 x 10 ⁷	36.3	1, 2
Abitibi-Price Inc.	Newsprint/purchased pulp	Alma	Oil and grease, suspended particulate matter, PAHs, resin acids	8.3 x 10 ⁷	NT- +++	1
Cascades Inc.	Corrugated boxes/ Kraft and thermo-mechanical pulp	Jonquière	Oil and grease, suspended particulate matter, resin acids, chlorinated organics	4.0 x 10 ⁷	NT- +	1

Table 1 continued.....

Abitibi-Price Inc.	Specialty paper products/purchased pulp	Jonquière	Oil and grease, suspended particulate matter, resin acids, PAHs	1.3×10^8	366.0	1, 2
Alcan Ltd. (Arvida facility)	Al ingots, alumina, specialty inorganic chemicals/Soderberg process	Jonquière	F, Al, Oil and grease, phenols, Hg, PAHs	6.5×10^7	NT- +++	1
Alcan Ltd. (La Baie facility)	Al ingots, precooked anodes/Soderberg process- precooked anodes	La Baie	F, Al, suspended particulate matter, PAHs	3.1×10^5	2.5	1, 2
Stone-Consolidated Inc.	Newsprint/thermom-echanical pulp	La Baie	Suspended particulate matter, oil and grease, resin acids, PCBs [†] , PAHs	6.2×10^7	NT- +	1

* (1) EIS (1992), (2) White et al. (1995a).

[†]Polychlorinated biphenyls

Extracts were filtered through a coarse sintered glass funnel, dried with anhydrous sodium sulfate (Mallinckrodt Specialty Chemicals, Mississauga, Ontario) and reduced to approximately 5 mL by rotary evaporation at 30°C (Büchi Laboratorium-Technik AG, Flawil/Schweiz, Switzerland). All extracts were taken to dryness under a gentle stream of ultrapure N₂ gas. Prior to resuspension, the mass of the extractable residue was determined gravimetrically. Final extracts were resuspended in dichloromethane (Sigma Chemical Co., St. Louis, MO) in preparation for gel permeation clean-up.

All labware was precleaned with pesticide grade acetone and hexane. Glassware was baked at 350°C to remove solvent traces and covered with pre-cleaned aluminum foil. Glass fibre filters and sodium sulfate were combusted at 450°C before use.

Gel Permeation Chromatography

Lipids and other high molecular weight substances (e.g. carotenoids, chlorophylls) that can interfere with the performance of the aqueous genotoxicity assay were removed via GPC (gel permeation chromatography) on Bio-Beads™ SX-3 (Bio-Rad Laboratories, Mississauga, Ontario). Although other methods (e.g. saponification, alumina cleanup) can be employed to removed lipids and other interfering substances, GPC is nondestructive and, other than molecular size, it makes no assumptions about the physical/chemical properties of the toxic substances present in the complex extract. The method employed is a variation on EPA method 3640 (USEPA, 1986) incorporating some of the modifications described by Czuczwa and Alford-Stevens (1989) and Engel (1985). Briefly, ≈50 g of Bio-Bead™ resin was permitted to swell overnight in 45% dichloromethane in hexane (Anachemia Science, Montreal) and packed into 35 cm (≈175 mL) glass columns (Corning, Richmond Hill, Ontario). Once settled, the column was washed with 100 mL of fresh solvent. Biota extracts were applied to pre-calibrated columns and eluted under gravity at 1 mL min⁻¹. The lipid fraction (80-90 mL) was collected for lipid content determination by gravimetric analysis. Sample collection was continued to a total elution volume of 250 mL. Samples were reduced to approximately 5 mL by rotary evaporation at 30°C and taken to dryness under vacuum at low temperature (Speedvac AS290 Concentrator, Savant Ltd., Farmingdale, NY). The weight of the final post-GPC residue was measured, an aliquot equivalent to 1 g of wet tissue was removed for PAH (polynuclear aromatic hydrocarbon) analyses, and the remaining extract was dissolved in high-purity DMSO (dimethyl sulfoxide) (Sigma Chemical Co., St. Louis, MO).

GPC columns were calibrated with a dichloromethane solution containing 100 mg mL⁻¹ corn oil, 2 mg mL⁻¹ pentachlorophenol (CAS # 87-86-5, Aldrich Chemical Co., Milwaukee, WI), 2 mg mL⁻¹ bis(2-ethylhexyl) phthalate (CAS # 117-81-7, Aldrich

Chemical Co., Milwaukee, WI), 0.04 mg mL⁻¹ perylene (CAS # 198-55-0, Aldrich Chemical Co., Milwaukee, WI) and 0.1 mg mL⁻¹ sulfur (CAS # 7704-34-9, Anachemia Science, Montreal). Flow conditions (under gravity) and lipid load were adjusted to optimize the separation of corn oil lipids from *bis*(2-ethylhexyl) phthalate. Optimal results were obtained with a flow rate of 1 mL min⁻¹ and a lipid load that did not exceed 250 mg. Elution of calibration compounds was monitored by absorbance at 254 nm (Beckman DU-70 UV-VIS spectrophotometer, Beckman Instruments, Palo Alto, CA). Dump volumes were adjusted to permit >85% removal of the lipids and >85% recovery of the *bis*(2-ethylhexyl) phthalate (USEPA, 1986). Dump volumes ranged from 80-90 mL with complete sulfur elution at approximately 160 mL.

SOS Chromotest Analyses

Genotoxicity was measured using the SOS Chromotest (Quillardet et al., 1982; Quillardet and Hofnung, 1985). The test involves incubation of *Escherichia coli* PQ37 (*lacΔU169 uvrA rfa sulA::Mud(Ap,lac) cts* PhoC) with the pure compound or complex mixture to be investigated. The *sulA::Mud(Ap lac)* fusion places the production of functional β-galactosidase under the express control of the SOS response pathway which is induced by DNA damaging agents (Walker, 1984). Post-exposure β-galactosidase activity reflects the genotoxicity of a given sample. Alkaline phosphatase activity provides an indirect measure of cell viability (Quillardet et al., 1982). Each extract was tested both in the presence and in the absence of a post mitochondrial supernatant (S9 fraction) obtained from Arochlor 1254 induced rat liver (Molecular Toxicology Products Inc., Annapolis, MD). S9 mixture was prepared following the method of Quillardet and Hofnung (1985). Final S9 concentration was 1% v/v. 4-Nitroquinoline-1-oxide (CAS # 56-57-5, Sigma Chemical, St. Louis, MO) and 2-aminoanthracene (CAS # 613-13-8, Sigma Chemical, St. Louis, MO) were used as positive controls.

The protocol employed is a variation on that described by Quillardet and Hofnung (1985). To permit rapid analysis of a large number of samples, the assay was carried out in microtiter plates and automated using the Biomek™ 1000 Automated Laboratory Workstation (Beckman Instruments, Palo Alto, CA) (see White et al., 1995c for details). Briefly, a 16 hour overnight culture (37°C) of *E. coli* PQ37 was diluted in fresh medium (Luria-Bertani (LB) broth + 20 µg per mL ampicillin) to an optical density (600 nm) of 0.05-0.075. The sample (or solvent blank), dissolved in 10 µL DMSO was added to 190 µL of diluted culture (plus S9 if desired) present in the first well of each dilution series and serially diluted to provide a total of six, 2-fold dilutions. Maximum tested concentrations were 20-25 equivalent g wet tissue assay mL⁻¹. Twelve control wells received PQ37

culture and a solvent blank. Each extract was tested in triplicate at the highest possible concentration range. The final background concentration of DMSO was 5% v/v.

Microplates were incubated for 3 hours at 37°C and subsequently centrifuged at 2000 g for 20 minutes. The supernatant was removed and the bacterial pellet resuspended in 100 µL of 200 mM (Tris[hydroxymethyl])-aminomethane (CAS # 77-86-1) pH 7.5 and 100 µL of cell lysis/enzyme assay buffer (40% v/v methanol (CAS # 67-56-1), 0.5% v/v toluene (CAS # 108-88-3), 10% v/v N,N-dimethyl formamide (68-12-2), 4 mg/mL 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal, CAS # 7240-90-6)), 1 mg/mL *p*-nitrophenyl phosphate (CAS # 4262-83-9)). Centrifugation and supernatant removal permitted the removal of colored substances that can contribute to the sample's optical density and complicate enzyme activity measurements. Plates were mixed for 60 seconds (Titertek microplate vortex, Flow Laboratories, Lugano, Switzerland) and incubated (37°C) for 60 minutes after which *initial* optical density readings (Titertek Multiskan MCC/340 Microplate Spectrophotometer) were taken (620 nm and 405 nm). Final optical density readings were taken 120 minutes after cell resuspension and lysis. Optical density values recorded at 620 nm reflect the activity of β-galactosidase, those recorded at 405 nm measure the activity of alkaline phosphatase. Calculation of net optical density values (120 min value - 60 min value) allowed the contribution of any remaining colored substances to be accounted for prior to data analysis.

Data Analyses

Induction of the SOS response pathway at a sample concentration *C*, denoted *R*(*C*), is the ratio of net (120 min - 60 min) β-galactosidase activity to net alkaline phosphatase activity. To account for the background induction of the *sulA* gene, a normalized SOS response induction factor was calculated as $IF = R(C)/R(0)$, where *R*(0) is the ratio of β-galactosidase to alkaline phosphatase activity averaged over the 12 controls which received blank solvent. A normalized induction factor that exceeded the upper 95% confidence limit of the control was regarded as a significant positive. Since normalized induction factor values are ratios of two means, confidence limits were calculated according to Welsch et al. (1988). Concentration response curves of normalized induction factor (IF) against sample concentration (equivalent g wet tissue per well) were inspected and the results qualitatively categorized as follows-

- 1) Negative- induction factor never exceeded the upper confidence limit of the control;
- 2) Marginal- induction factor exceeded the upper confidence limit of the control at one or two concentrations only;

- 3) Positive- induction factor exceeded the upper confidence limit of the control at a minimum of three doses;
- 4) Erratic- induction factor exceeded the control at several concentrations, but the concentration-response relationship was highly erratic and could not be interpreted.

Every effort was made to remove erratic responses from the data set. All samples that produced erratic responses, especially where growth inhibition was substantial, were reanalyzed at one-tenth and/or one-hundredth the original concentration range. Genotoxic potency values were calculated for each sample that elicited a significant positive response. The SOS response inducing potency (SRIP) is the initial slope of the concentration response curve expressed in SOS Induction Factor units per equivalent g of wet tissue. Lipid content values were used to calculate lipid corrected SRIPs, the SOS response inducing potency in SOS Induction Factor units per equivalent mg of extractable lipid.

When concentration response curves were distinctly nonlinear, exhibiting zero-order kinetics at high sample concentration, they were analyzed by iterative, non-linear regression employing a least squares loss function (SAS Institute, 1988). In these cases the SRIP was calculated according to Langevin et al. (1992). When the range of concentrations tested did not result in a full hyperbola, the SRIP was determined by ordinary, least squares linear regression.

Polycyclic Aromatic Hydrocarbon (PAH) Analyses

For PAH analyses, an aliquot of each post-GPC extract equivalent to 1 g of wet tissue was dissolved in 100 μ L pesticide-grade benzene (Anachemia Science, Montreal). Each sample was spiked with d₁₀-fluoranthene (Supelco Canada, Oakville, Ontario), the internal standard. The concentrations of 21 PAHs were measured via gas chromatography (GC)/mass spectrometry (MS) (HP 5890 Series II GC with HP 5971A MS, Hewlett-Packard Canada, Ltd., Mississauga, Ontario) in SIM (selected ion monitoring) mode. A list of the PAHs analyzed is provided in Table 2. 1 μ L of each sample was injected *on column* with the following GC/MS conditions:

Column- J&W DB-5 30 m x 0.25 mm (Alltech Associates Inc., Guelph, Ontario);
 Mass Range- 127-302 amu;
 Temperature program- 80°C for 2 min,
 80°-230°C at 10°C per min, 230°-300°C at 20°C min⁻¹;
 Carrier gas- He at 8 psi, 80°C with constant flow.

Table 2. Polycyclic aromatic hydrocarbons investigated in this study. All require S9 metabolic activation.

Compound	CAS #*	Molecular Weight (g/mole)	Genotoxic?/ Carcinogenic?†	Detected in Collected Samples?
Naphthalene (NAPH)	91-20-3	128.2	0/0	No
Acenaphthylene (ACENY)	208-96-8	152.2	NA	No
Acenaphthene (ACEN)	83-32-9	154.2	0/NA	No
Fluorene (FLU)	86-73-7	166.2	0/I	No
Phenanthrene (PHEN)	85-01-8	178.2	++?/I	Yes
Anthracene (ANTH)	191-26-4	178.2	+/0	Yes
Fluoranthene (FLUOR)	206-44-0	202.3	++/0	Yes
Pyrene (PYR)	129-00-0	202.2	++?/0	Yes
Benzo(c)phenanthrene (B(c)P)	195-19-7	228.3	+/I	Yes
Benz(a)anthracene (B(a)A)	56-55-3	228.3	++/++	Yes
Chrysene (CHRY)	218-01-9	228.3	++?/+	Yes
Benzo(j)fluoranthene (B(bjk)F)	205-82-3	252.3	+/++	Yes ^o
Benzo(b)fluoranthene	205-99-2	252.3	++?/++	--
Benzo(k)fluoranthene	207-08-9	252.3	+/++	--
Benzo(a)pyrene (B(a)P)	50-32-8	252.3	+++ / ++	Yes
Indeno(1,2,3-cd)pyrene (I(123cd)P)	193-39-5	276.3	+ / ++	Yes
Dibenz(a,h)anthracene (DB(ah)A)	53-70-3	278.3	++ / ++	Yes
7,12-Dimethylbenz(a)anthracene (DMBA)	39834-38-3	256.3	+ / ++	Yes

Table 2 continued.....

3-Methylcholanthrene (3MC)	56-49-5	268.3	+ / ++	Yes
Benzo(<i>ghi</i>)perylene (B(<i>ghi</i>)P)	191-24-2	276.3	+ / I	Yes
Dibenz(<i>a,h</i>)pyrene (DB(<i>ah</i>)P)	189-64-0	302.4	+ ? / ++	No
Dibenz(<i>a,l</i>)pyrene (DB(<i>al</i>)P)	191-30-0	302.4	+ / ++	Yes
Dibenz(<i>a,i</i>)pyrene (DB(<i>ai</i>)P)	189-55-9	302.4	+ / ++	No

*Chemical Abstracts Service registry number.

†Based on information from IARC (1983); Hermann (1981); Sakai et al. (1985); LaVoie et al. (1980); Mersch-Sundermann et al. (1992); Mersch-Sundermann et al. (1993); Gold et al. (1984); Gold et al. (1986). Genotoxicity symbols are: 0 - no evidence of genotoxicity, + - indicates positive response on prokaryotic short-term tests, ++ - indicates positive response on both prokaryotic and eukaryotic short-term tests, ? - indicates conflicting results reported in the literature.

Carcinogenicity symbols are: 0 - no evidence of carcinogenicity, I - inadequate evidence for evaluation, + - limited evidence of carcinogenicity in experimental animals, ++ - sufficient evidence of carcinogenicity in experimental animals. NA indicates data not available.

‡Results reported as the sum of benzo(*b*)fluoranthene, benzo(*k*)fluoranthene and benzo(*j*)fluoranthene.

RESULTS

A total of 13 samples were collected from the 9 sites indicated on Figure 1. With the exception of one sub-sample from Baie du Moulin à Baude and one sub-sample from Anse à la Barque, all samples elicited a significant, direct-acting positive response on the SOS Chromotest. In many cases the SOS induction factor values observed rivaled those obtained in our previous examinations of industrial effluent extracts. Less than 10% of the 150 effluent extracts examined by White et al. (1995a) elicited an SOS induction factor above 2.5. Almost one-third of the bivalve extracts examined here elicited SOS induction factors above 2.5. Several elicited responses above 3.0. Figure 2 includes concentration-response plots for 4 samples that elicited strong, positive responses. In each case the maximum induction factor obtained exceeded or approached 3.0. Table 3 provides a summary of the SOS Chromotest (without S9 metabolic activation) and PAH results. Linear regression analyses indicated that there is a weak effect of lipid content on both PAH contamination (ng g tissue^{-1}) and sample genotoxicity (IF g tissue^{-1}). For this reason the values presented are expressed both per equivalent unit of wet tissue and per equivalent unit of extractable lipid. All inter-site comparisons and subsequent data analyses were performed on the lipid corrected genotoxicity and PAH concentration values.

The results indicate that SRIP values range from a high of 0.272 (IF mg lipid^{-1}) at Anse à la Grosse Roche to a low of 0.023 (IF mg lipid^{-1}) at Anse St-Étienne-West. Samples collected at three sites in Anse St-Étienne indicate ≈ 2 -fold variability in potency within one bay. In general, the samples taken outside the Fjord (e.g. Baie Ste-Catherine and Baie du Moulin à Baude) tend to elicit weaker responses than those collected in the upper regions of the Fjord (e.g. Anse St-Jean, Petit Saguenay, Anse St-Louis). Examination of PAH concentration values reveals a similar pattern. Similar differences in mollusc PAH contamination between Saguenay Fjord and the St. Lawrence estuary samples were described by deLadurantaye et al. (1991). Samples from Anse à la Grosse Roche were found to be highly genotoxic, as well as highly contaminated with PAHs. Table 4 provides a detailed summary of the PAH results. All the samples examined, including the matrix blank, contained detectable levels of genotoxic PAHs. *Mya arenaria* from Anse à la Grosse Roche contained PAH levels 25 times higher than the penultimate value. In addition, samples from Anse à la Grosse Roche contained high levels of several known carcinogens (see Table 2).

Britvic and Kurelec (1986), Kurelec and Krca (1987) and Anderson and Döös (1983) determined that bivalve molluscs have a limited capacity to activate PAHs such as B(a)P to bacterial mutagens. Therefore, we expected bivalves to accumulate progenotoxins that can

Figure 2. SOS Chromotest concentration response plots for dichloromethane extracts of *Mya arenaria* collected from four locations in the Saguenay Fjord. Mean SOS Induction factors values are based on concentrations tested in triplicate. Error bars are one standard error of the mean. Where error bars are not shown they were smaller than the plotting symbol. All results shown were obtained in the absence of S9 metabolic activation. The upper 95% confidence limit of the solvent control was calculated according to Welsch et al. (1988).

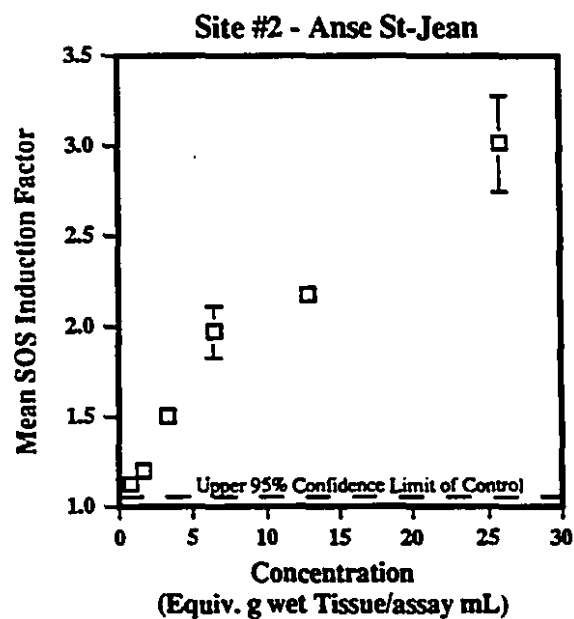
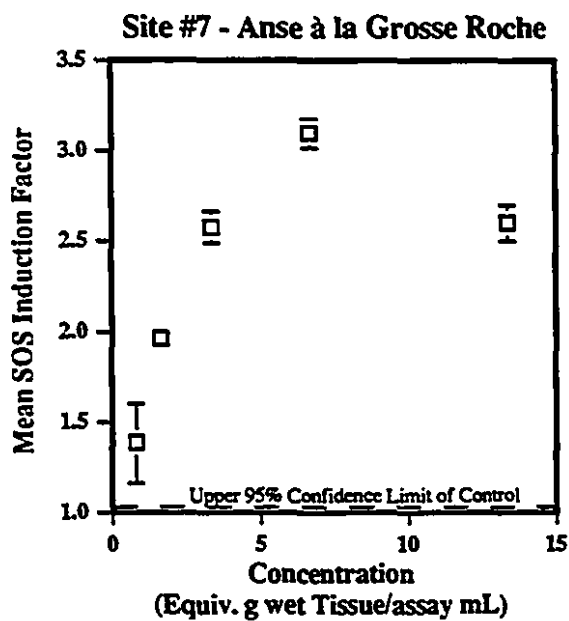
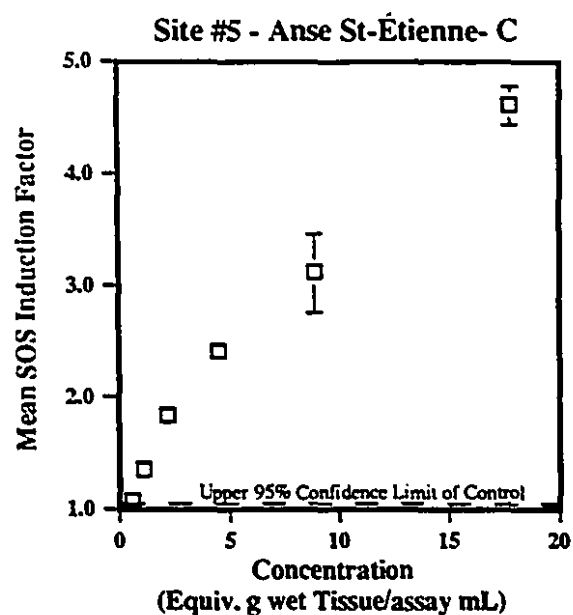
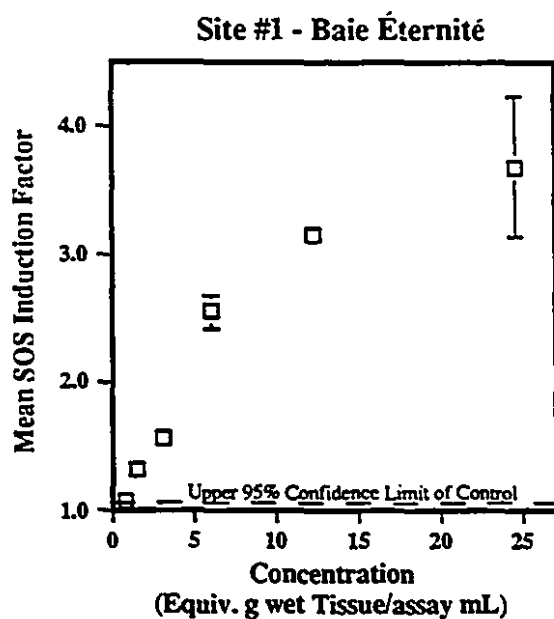


Table 3. SOS Chromotest (without S9 metabolic activation) and PAH residue results. Where several subsamples were examined (7 of 13 samples), values shown are arithmetic means. Three replicate samples were collected at Anse St-Étienne - East, West and Center. The matrix blank contained a mixture of freshwater invertebrates collected from a site that receives no direct industrial input. The solvent blank was 250 mL of dichloromethane. All samples elicited a significant positive response with the exception of one subsample from Baie du Moulin à Baude and one subsample from Anse à la Barque. The matrix blank elicited a marginal response with 1 of the 6 tested concentrations exceeding the upper confidence limit of the solvent control. ND indicates not detected.

Site	Sample	SRIP Values			Total PAH Values	
		Lipid (% wet wt.)	IF g Tissue ⁻¹	IF mg Lipid ⁻¹	ng g Tissue ⁻¹	µg g Lipid ⁻¹
Baie Éternité	<i>Mya</i>	0.64	0.210	0.0382	57.8	9.03
Anse St-Jean	<i>Mya</i>	0.22	0.268	0.123	37.3	16.66
Anse Petit Saguenay	<i>Mya</i>	0.43	0.349	0.0812	53.6	12.46
Anse Île St-Louis	<i>Mya</i>	0.67	0.581	0.0963	54.0	7.96
Anse St-Étienne- E	<i>Mya</i>	0.56	0.200	0.0491	37.0	7.86
Anse St-Étienne- W	<i>Mya</i>	0.55	0.103	0.0230	33.9	6.22
Anse St-Étienne- C	<i>Mya</i>	0.71	0.253	0.0350	40.6	6.09
Baie Ste-Catherine	<i>Mytilus</i>	0.38	0.204	0.0537	16.8	4.42
Anse à la Grosse Roche	<i>Mya</i>	0.22	0.600	0.272	1399.6	636.19
Anse à la Barque	<i>Mya</i>	0.52	0.281 [†]	0.0425	16.4	3.49
Anse à la Barque	<i>Mytilus</i>	0.55	0.159	0.0289	23.8	4.33
Baie du Moulin à Baude	<i>Mya</i>	0.24	0.0361 [§]	0.0328	4.4	4.00

Table 3 continued.....

Baie du Moulin à Baude	<i>Mytilus</i>	0.56	0.292	0.0521	1.0	0.18
Matrix Blank	Mixed*	0.82	-----	-----	20.3	2.48
Solvent Blank	-----	-----	ND	-----	ND	ND

*Included freshwater amphipods (Gammaridae) and insect nymphs (predominantly Aeshnidae and Phryganeidae) from L. Memphremagog.

†One subsample elicited a marginal response (one concentration exceeded solvent control).

§One subsample failed to elicit a positive response.

Table 4. The concentrations (ng g wet tissue⁻¹) of identified PAHs in extracts of *Mya arenaria* and *Mytilus edulis* from the Saguenay Fjord. Values shown are rounded to the nearest 0.1 ng. Abbreviations are the same as in Table 2. Values can be converted to µg g lipid⁻¹ using the lipid content values presented in Table 3.

Site	Sample	PHEN	ANTH	FLUOR	PYR	B(c)P	B(a)A	CHRY	B(bjk)F	DMBA	B(a)P	3-MC	I(123cd)P	DB(ah)A	B(ghi)P	DI(1a)P
Île d'Orléans	<i>Mya</i>	0.0	3.0	13.0	10.0	1.0	3.0	16.9	6.9	0.0	2.0	0.0	0.0	0.0	2.0	0.0
St-Jean	<i>Mya</i>	1.0	0.5	6.5	5.5	0.0	2.0	9.9	7.0	0.0	2.5	0.0	1.0	0.0	1.5	0.0
Petit Saguenay	<i>Mya</i>	0.0	2.0	10.9	7.9	1.0	4.0	15.9	7.9	0.0	2.0	0.0	0.0	0.0	2.0	0.0
St-Louis	<i>Mya</i>	1.0	1.0	11.2	8.3	1.0	2.0	16.1	10.7	0.0	1.0	0.0	0.0	0.0	2.0	0.0
St-Étienne-E	<i>Mya</i>	1.0	0.5	6.8	4.9	0.0	1.9	10.2	7.8	0.0	2.4	0.0	0.0	0.0	1.4	0.0
St-Étienne-W	<i>Mya</i>	0.0	1.0	4.5	4.0	0.5	2.0	11.5	6.5	0.0	2.0	0.0	0.0	0.0	2.0	0.0
St-Étienne-C	<i>Mya</i>	0.0	1.5	8.4	6.0	0.0	2.0	12.4	5.9	0.0	2.0	0.0	0.0	0.0	2.5	0.0
Ste-Catherine	<i>Mya</i>	0.0	2.0	4.0	3.0	0.0	0.0	4.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0
Grosse Roche	<i>Mya</i>	0.0	0.0	3.9	2.9	8.9	5.8	6.8	4.8	1158.3	4.8	32.8	164.1	4.8	1.9	0.0
Barque	<i>Mya</i>	0.0	0.9	1.5	2.9	0.0	1.0	5.3	3.4	0.0	1.0	0.0	0.0	0.0	0.5	0.0
Barque	<i>Mytilus</i>	0.0	2.0	5.0	4.0	0.0	1.0	6.0	5.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0
Baude	<i>Mya</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.5	0.0	1.0	0.0	2.0	0.0
Baude	<i>Mytilus</i>	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Matrix Blank	Mixed*	1.0	0.0	7.7	6.8	0.0	0.0	1.9	2.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Solvent Blank	—	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*Included freshwater amphipods (Gammaridae) and insect nymphs (predominantly Aeshnidae and Phryganeidae).

be activated *in vitro* by mammalian liver microsomes. However, for all samples examined, the addition of S9 resulted in a reduction of genotoxicity. On average, the potency values measured in the absence of S9 were 6 to 10 fold higher than those measured in the presence of S9. No extracts elicited a stronger response in the presence of S9 activation.

Previous examinations of industrial wastewaters discharged directly into the Saguenay river (see Table 1) confirm that genotoxic substances (both known and unknown) are being released. For example, the large Soderberg Al smelter at Jonquière (Arvida facility in Table 1) discharges almost 2 kg of PAHs per day including ≈ 5 g of B(a)P (EIS, 1992). Direct release into the river suggests that sampling sites closer to the effluent source should yield samples with higher genotoxic potency and higher tissue concentrations of PAHs. Although some samples collected in the lower portions of the Fjord provided lower SRIP values (e.g. Anse à la Barque and Baie du Moulin à Baude), this is not a general trend. The site that is closest to the presumed industrial sources (Baie Éternité) yielded samples that are no more genotoxic than those collected much further downstream (e.g. Anse à la Barque and Anse St-Étienne). In addition, some samples taken further downstream at Anse St-Jean, Anse Île St-Louis, Anse Petit Saguenay and Anse à la Grosse Roche yielded higher SRIP values than those collected at Baie Éternité. PAH levels in Baie Éternité samples were also lower than samples collected further downstream at Anse St-Jean, Anse Île St-Louis, Anse Petit Saguenay and Anse à la Grosse Roche. In the absence of a clear trend of decreasing tissue genotoxicity and PAH concentration with increasing distance from the presumed industrial sources, we investigated relationships between sample genotoxicity and demographic variables. White and Rasmussen (1995b) determined that most of the genotoxic activity in surface waters can be accounted for by domestic rather than industrial inputs. They further demonstrated that surface water genotoxicity and mutagenicity levels can be predicted solely from demographic and hydrologic information. Figure 3 illustrates the relationship between genotoxic potency and the number of inhabitants within 5 km of the sampling site shoreline. Figure 3a illustrates that for seven of the nine sites examined there is a significant trend ($r^2 = 0.80$, $p < 0.007$), suggesting that local pollution sources contribute to the direct-acting genotoxicity of tissue extracts. The results further suggest that Anse Île St-Louis and Anse à la Grosse Roche are receiving direct-acting genotoxic substances from other sources in addition to local, domestic discharges. Figure 3b indicates that there is also a relationship between S9 activated genotoxicity and the number of inhabitants within 5 km of the shoreline ($r^2 = 0.53$, $p < 0.03$).

We also identified a significant relationship ($r^2 = 0.71$, $p < 0.005$) between genotoxic potency without S9 (IF mg lipid⁻¹) and PAH concentration ($\mu\text{g g lipid}^{-1}$). This relationship is shown in Figure 4a. It should be noted that this relationship is substantially weakened if

Figure 3. The relationship between the genotoxic potency of tissue extracts (IF Units per equivalent mg lipid) and the number of inhabitants within 5 km of the sampling site shoreline. Population data are from the 1991 census of Canada (Statistics Canada, 1993) and 1:50 000 topographic maps. Where several samples were taken from a given site (e.g. Baude, Barque and St-Étienne) potency values presented are averages. A- Genotoxic potency without S9 activation vs. Shoreline Population. The plotted line represents the least-squares linear regression fit for seven of the nine sites ($r^2 = 0.80$, F Ratio = 19.6, $p < 0.007$). Diamond-shaped plotting symbols were not included in the regression analysis. The regression function is $\text{Log Genotoxic Potency (without S9)} = -1.65 + (0.19 * \text{Log Shoreline Population})$. B- Genotoxic potency with S9 activation vs. Shoreline Population. The plotted line represents the least-squares linear regression fit for all nine sites ($r^2 = 0.53$, F Ratio = 7.9, $p < 0.03$). The regression function is $\text{Log Genotoxic Potency (with S9)} = -2.38 + (0.24 * \text{Log Shoreline Population})$. In both cases the data were log transformed to equalize the variance over the range of observations and meet the normality assumption of least squares regression (Ricker, 1973).

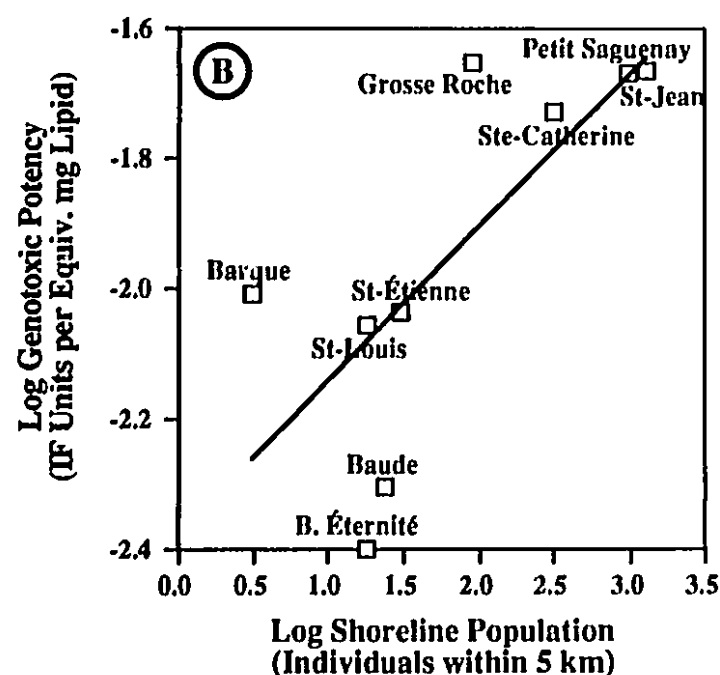
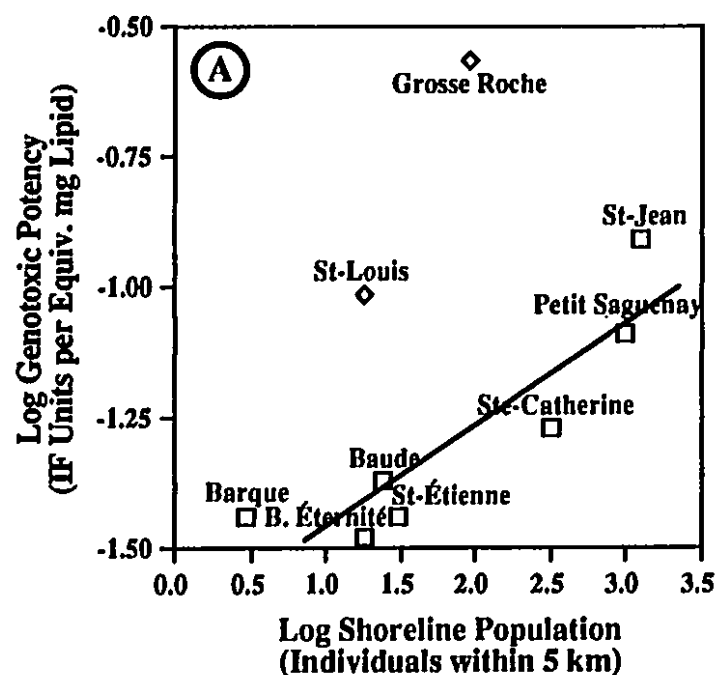
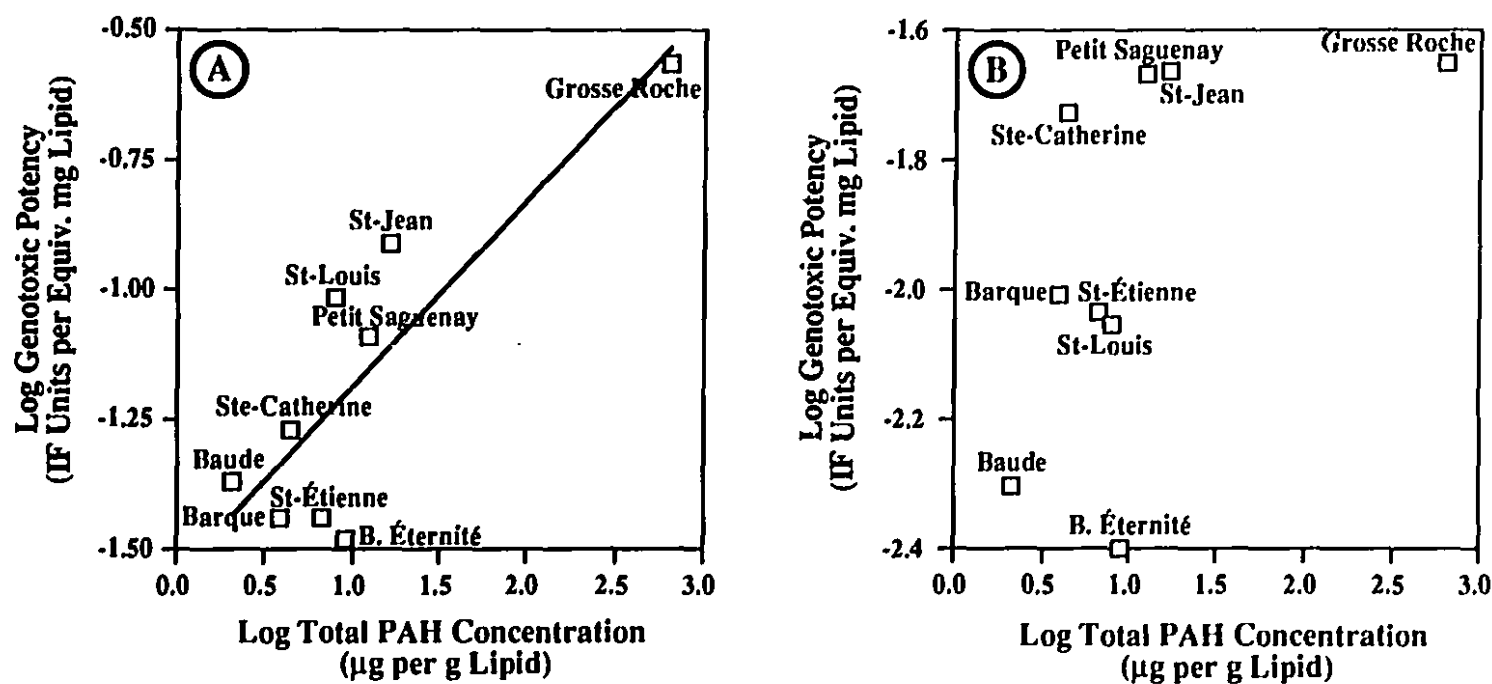


Figure 4. The relationship between the genotoxic potency of tissue extracts (IF Units per equivalent mg lipid) and the tissue PAH concentration (μg PAH per g lipid). Total PAH concentrations are sums of the 21 measured PAHs (see Table 2). Where several samples were taken from a given site (e.g. Baude, Barque and St-Étienne) values presented are averages. A- Genotoxic potency without S9 activation vs. Total PAH concentration. The plotted line represents the least-squares linear regression fit ($r^2 = 0.71$, F Ratio = 17.2, $p < 0.005$). The regression function is $\text{Log Genotoxic Potency (without S9)} = -1.56 + (0.36 * \text{Log PAH Concentration})$. B- Genotoxic potency with S9 metabolic activation vs. Total PAH concentration. No significant relationship was found ($r^2 = 0.28$, $p = 0.15$). In both cases the data were log transformed to equalize the variance over the range of observations and meet the normality assumption of least squares regression (Ricker, 1973).



Anse à la Grosse Roche data are removed ($r^2 = 0.51$, $p < 0.03$, Saguenay Fjord sites only). Figure 4b illustrates the relationship between S9 activated genotoxic potency and PAH concentration. Linear regression analyses failed to reveal a statistically significant relationship. However, the results do suggest the presence of a non-linear trend reaching zero-order kinetics at high PAH concentrations.

Although aluminum smelters release large amounts of genotoxic PAHs (IARC, 1984; Table 1), the aforementioned absence of a clear downstream trend in PAH levels do not support contamination via *direct* discharges into the Fjord. Smith and Levy (1990) noted that in addition to aqueous emissions, atmospheric emissions of PAHs from Aluminum smelters in the Saguenay-Lake St. Jean region exceed 100 tonnes per year. If these airborne emissions are contaminating bivalves that inhabit small bays along the Fjord, we would expect to find a relationship between PAH levels in the bivalves and drainage basin area. If Anse à la Grosse Roche is removed from the data set, there is indeed a significant relationship ($r^2 = 0.64$, F Ratio = 10.9, $p < 0.02$) between PAH contamination (μg Total PAH per g lipid) and drainage basin area (km^2) ($\text{Log PAH in } \mu\text{g g lipid}^{-1} = 0.48 + (0.23 * \text{Log Drainage Basin Area in km}^2)$). Again the evidence indicates that Anse à la Grosse Roche is receiving local, point sources input(s). PAH contamination is not related to either shoreline population or total drainage basin population.

DISCUSSION

Many researchers have employed chemical analyses to detect genotoxins, mutagens and carcinogens in aquatic fauna (e.g. Marvin et al., 1994; Smith et al., 1984; Mix and Schaffer, 1979; Dunn and Stich, 1975; Andelman and Suess, 1970). However, there are distinct advantages to employing a bioassay system for the detection of genotoxins in environmental samples. Environmental monitoring by direct chemical analyses requires *a priori* knowledge of contaminant identity. Even after successful detection and identification, detailed chemical analysis is limited by its inability to predict the (geno)toxicity of complex mixtures (Brouwer et al., 1990; Samoiloff, 1983; Houk, 1992). In addition, the (geno)toxicity of unaltered aqueous samples or organic extracts can rarely be accounted for by the concentration of identified (geno)toxins (Ellis et al., 1982; Hendricks et al., 1994). Bioassays do not require any knowledge about the putative (geno)toxins, and are capable of integrating the effects of all mixture components. Subsequent bioassay-directed fractionation procedures (e.g. see Alfheim et al., 1984) can then be employed to isolate and identify (geno)toxins. Despite these advantages, few published studies have attempted to use bioassays to investigate the accumulation of genotoxic substances in aquatic biota that inhabit regions contaminated with industrial and municipal wastes. Table 5 lists published research efforts that employed bioassays to detect genotoxins or mutagens in aquatic biota. All of the studies listed (except this one) employed the *Salmonella*/mammalian microsome assay for mutagenicity detection. Although the assay has been used successfully, most of the researchers listed (e.g. Parry et al., 1980; Parry et al., 1976; Sparks et al., 1981; Frezza et al., 1982; Marvin et al., 1994) have described problems caused by the presence of interfering substances in biota extracts. The major goal of this work was to determine the suitability of the SOS Chromotest for the study of aquatic biota that inhabit regions known to receive genotoxic wastes.

The SOS Chromotest results obtained (Figure 2, Table 3) indicate that bivalve molluscs inhabiting the Saguenay Fjord are indeed contaminated with genotoxic substances. This may not be surprising for several reasons. The industries listed in Table 1 are known to discharge genotoxic substances into the river and Fjord (White et al., 1995a; EIS, 1992; Smith and Levy, 1990). Fjord sediments are known to be contaminated with genotoxic and carcinogenic PAHs of pyrolytic origin (Martel et al., 1987; Martel et al., 1986; Smith and Levy, 1990). Finally, bivalve molluscs are renowned for their limited ability to metabolize and excrete genotoxic PAHs (McElroy et al., 1989; James, 1989; Stegman and Lech, 1991). While recent evidence indicates that bivalves can oxidize PAHs such as B(a)P, the rate of oxidation is very low (Stegman, 1985) and not (or poorly)

Table 5. Summary of published research that used short-term, microbial bioassays to investigate the presence of genotoxins or mutagens in tissues of marine and freshwater biota.

Biota Examined	Extraction Procedure	Bioassay Employed	Result	Source†
Mussel (<i>Mytilus edulis</i>) tissues	Ethanol extract	Fluctuation assay with several strains of <i>E.coli</i> , <i>S. typhimurium</i> and <i>S. cerevisiae</i>	Several + responses (some >10 fold control) on samples from industrialized areas	1
Mussel (<i>Mytilus edulis</i>) tissues	Ethanol extract	Fluctuation assay with several strains of <i>S. typhimurium</i> and <i>E. coli</i>	Several + responses on samples from industrialized areas	2
Mussel (<i>Mytilus edulis</i>) tissues	Ethanol and nitric/perchloric acid extract	Fluctuation assay with several strains of <i>E.coli</i> , <i>S. typhimurium</i> and <i>S. cerevisiae</i>	Several + responses (some >10 fold control) on samples from industrialized areas	3
Oysters (<i>Crassostrea virginica</i>), Sea squirts (<i>Mogulla sp.</i>), Shrimps (<i>Peneaus duorarum</i>)	2-propanol extraction followed by XAD adsorption	Plate incorporation assay with <i>S. typhimurium</i> TA98, TA100, TA1535, TA1537 and TA1538	Some marginal responses on Sea squirt (residential bay) and shrimp (market purchased) extracts	4
Sea Trout (<i>Nibea mitsukurii</i>) liver	Ether/methanol Soxhlet extract	Plate incorporation assay with <i>S. typhimurium</i> (TA98, TA100 + TA1537) <i>B. subtilis</i>	Marginal responses on samples collected near pulp mill discharge	5
Mussels (<i>Mytilus sp.</i> , market purchased)	Ethanol/Soxhlet extract	Plate incorporation assay with <i>S. typhimurium</i> (TA 98 and TA100) <i>Saccharomyces cerevisiae</i> and <i>Schizosaccharomyces pombes</i>	Several + responses on samples from heavily polluted areas (Venice lagoon)	6

Table 5 continued.....

Shrimps (<i>Peneaus semisulcatus</i>), Clams (<i>Circenita callipyga</i>)	Various methods explored	Fluctuation assay with <i>S. typhimurium</i> TA98 and <i>E. coli</i> WP ₂	Strong + response with ethanol extract	7
Mussels (<i>Mytilus edulis</i>), Limpets (<i>Patella vulgata</i>)	Nitric acid extract	Fluctuation assay with <i>S. typhimurium</i> TA98 and TA100	Strong + response following oil spill	8
Mussel Tissue (unspecified)	Acetone/blue cotton extract	Plate incorporation assay with <i>S. typhimurium</i> TA98	Strong + response (>10 fold control) in industrialized area	9
Various marine molluscs (<i>Chamelea gallina</i> , <i>Ruditapes decussatus</i> , <i>Crassostrea gigas</i>)	Ethanol extraction followed by XAD adsorption	Plate incorporation assay with 4 strains of <i>S. typhimurium</i> and 5 strains of <i>E. coli</i>	Several + responses suggesting polar, oxidative mutagens	10
Zebra mussels (<i>Dreissina polymorpha</i>)	Dichloromethane extraction followed by alumina clean-up	Plate incorporation assay with <i>S. typhimurium</i> TA100 and YG1029	Strong + response at PAH contaminated sites (+S9 only)	11
Soft-shell clams (<i>Mya arenaria</i>) and Blue Mussels (<i>Mytilus edulis</i>)	Dichloromethane extract followed by GPC clean-up.	Microplate version of the SOS Chromotest (<i>E. coli</i> PQ37)	Strong + response for PAH contaminated samples	12

†(1) Parry et al. (1976), (2) Parry and Al-Mossawi (1979), (3) Parry et al. (1980), (4) Sparks et al. (1981), (5) Kinac et al. (1981), (6) Frezza et al. (1982), (7) Al-Mossawi et al. (1982), (8) Kadhim and Parry (1984), (9) Kira et al. (1989), (10) Rodriguez-Ariza et al. (1992), (11) Marvin et al. (1994), (12) This study.

inducible by exposure to well known MFO (mixed function oxidase) inducers. Measurements of digestive gland MFO activity in *Mya arenaria* from the Saguenay Fjord confirm that levels are indeed low ($1-3 \text{ pmoles min}^{-1} \text{ mg protein}^{-1}$ as 7-ethoxyresorufin-*O*-deethylase activity) and show no signs of induction at contaminated sites (Gagné, pers. comm.).

However, there are numerous problems with the conventional view that bivalves in the Fjord are accumulating industrial genotoxins emitted by industries located many kilometres upstream. First and foremost, the upstream industries are recognized for their emission of polycyclic aromatic hydrocarbons. PAHs identified in effluents from these industries require metabolic activation for detection in bacterial assay systems (Heflich, 1991; IARC, 1983). While the bivalve samples examined do contain progenotoxic PAHs of pyrolytic origin, the addition of a metabolic activation mixture reduced the potency of all sample extracts. Thus, it appears that the observed genotoxicity is primarily direct-acting, with the concentrations of progenotoxic PAHs being insufficient to cause an increase in genotoxicity when S9 is added. Therefore, although the bivalves are contaminated with PAHs, the detected PAHs cannot account for the observed SOS genotoxicity. It is possible that the upstream industries are emitting direct-acting substances that accumulate in the lower reaches of the Fjord. While chemical analyses of industrial effluents failed to detect any specific direct-acting substances, White et al. (1995a) noted that these industries do emit direct acting genotoxins that have yet to be characterized. Other researchers have also detected direct acting genotoxins in bivalve tissues. For example, Rodriguez-Ariza (1992) demonstrated that marine bivalves from contaminated sites off the coast of Spain accumulate direct-acting substances that modify DNA via oxidative mechanisms. The observed relationship between direct-acting genotoxicity and PAH contamination suggests that the putative direct-acting substances and the detected PAHs have similar physical-chemical properties. Therefore, although PAHs alone cannot account for the observed SOS genotoxicity, PAHs may serve as an effective tracer of genotoxins accumulated by bivalves.

Secondly, although the upstream industries may release direct-acting substances, the results obtained do not support contamination of bivalves with substances discharged directly into the Fjord as aqueous industrial emissions. Although Martel et al. (1986) and Martel et al. (1987) confirmed that genotoxic PAHs present in Fjord sediments appear to be pyrolytic PAHs discharged by the upstream aluminum smelters, they also determined that a substantial fraction of the released PAHs are removed from the water column long before they reach areas inhabited by marine bivalves. In addition to being deposited behind the hydroelectric dams between Alma and Chicoutimi (Schafer et al., 1990), PAH-laden

particles carried in the water column are deposited where the river meets the Fjord (sedimentation rate = 5 cm yr^{-1} - Smith and Walton, 1980). Therefore, industrial genotoxins reaching the lower regions of the Fjord would need to be sufficiently soluble and, as a result, not strongly sorbed to particulate matter. White et al. (1995d) demonstrated that genotoxins released by pulp and paper mills are much less likely to be sorbed to particulate matter ($K_d \approx 10^2 - 10^4$) than those emitted by Al smelters ($K_d \approx 10^6 - 10^7$). Thus, even in the absence of a downstream trend of decreasing genotoxicity, we cannot eliminate industrial contributions.

The results obtained further suggest that both genotoxins and PAHs may be reaching the lower regions of the Fjord via airborne transportation. The airborne route is supported by the results of Smith and Levy (1990). Smith and Levy calculated that between 1937 and 1980 airborne emissions of PAHs from aluminum smelters in the Saguenay-Lake St. Jean region resulted in annual depositions on the drainage basin of about 1000 tonnes. Only about 1% of this annual PAH load actually enters the Fjord. As a result the Fjord drainage basin contains an estimated reservoir of $\approx 40\,000$ tonnes of PAHs that have accumulated since 1938. Most of the PAHs detected in the bivalve samples contain 4 or more condensed rings (e.g. PYR, CHRY, B(a)P, B(a)A, FLUOR, B(ghi)P- see Table 4). This suggests that the source employs a high temperature combustion process (Prahl and Carpenter, 1983) such as that used in aluminum smelting ($\approx 1000^\circ\text{C}$, IARC, 1984).

Finally, the results presented in Figure 4 suggest that local municipal discharges contribute to the genotoxic contamination of *Mya arenaria* and *Mytilus edulis*. This empirical result is supported by the recent works of White and Rasmussen (1995b), Hoffman et al. (1984) and Matsui (pers. comm.). White and Rasmussen (1995b) demonstrated that the majority of direct-acting genotoxins entering surface waters of the St. Lawrence-Great Lakes system can be accounted for by domestic inputs. Hoffman et al. (1984) determined that a substantial fraction of the PAHs that enter surface waters, are from roadway runoff, commercial and residential area runoff and sewage treatment discharges. These sources likely contain direct-acting substances present in diesel emissions (Saleem et al., 1984). Matsui (pers. comm.) recently confirmed that human faeces and urine are important contributors to the genotoxicity of domestic wastewaters. Thus, genotoxins accumulated by bivalves in the Saguenay Fjord likely include substances discharged in sanitary wastes as well as substances contained in roadway runoff.

It is interesting to note that we were unable to identify any local point sources that contribute to the high contamination of samples from Anse à la Grosse Roche. However, the area is known to be treacherous and difficult to navigate. It is possible that the area is contaminated with fuel oil released from wrecked ships. Fuel oils, especially diesel fuels,

are known to containing genotoxic PAHs (Woo and Arcos, 1981). In addition, although the matrix blank contained detectable levels of PAHs, it did not contain any 5- or 6-ring PAHs. Smaller 3- and 4-ring PAHs often indicate contamination via fossil fuels and not high temperature pyrolysis (Prah and Carpenter, 1983). Although the site from which the matrix blank was collected does not receive any direct industrial input, it does receive heavy boat traffic.

This study has demonstrated the utility of the SOS Chromotest for environmental monitoring of aquatic biota. The bioassay has many other advantages in addition to its ability to accept biological samples. Survival of the tester strain is not required. The results can be obtained in a single working day. Sample sterility is not usually required. The organism responds to a wide range of DNA damage scenarios (Elespuru, 1987; Houk and deMarini, 1988). Bacteriostatic effects can be simultaneously monitored and very high daily throughput (> 50 samples day⁻¹) can be achieved with the microplate version of the assay (White et al., 1995c). The results obtained clearly demonstrate that *Mya arenaria* and *Mytilus edulis* in the Saguenay Fjord are contaminated with direct acting genotoxins. Although detected PAHs cannot account for the the direct acting responses, the putative genotoxins appear to have similar properties. These similarities make PAHs an effective tracer of genotoxins in both the biota and the watershed. While the results provide some support for contamination by pyrolytic PAHs from upstream Al smelters, they provide little support for contamination via direct aqueous discharge. Although the results provided empirical evidence to support contamination by genotoxins of domestic origin, and genotoxins transported in the atmosphere, the putative substances remain to be identified. They likely include a mixture of substances from industrial wastes, sanitary wastes and roadway runoff. The industrial contributions may include aqueous genotoxins discharged by pulp and paper facilities. Contributions from Al smelters are likely contained in airborne emissions. Determining the identity, precise origins and biological effects of genotoxins in bivalves that inhabit the Saguenay Fjord seems a promising area for further research.

ACKNOWLEDGMENTS

We gratefully acknowledge the support and assistance of several people. John Hepworth for assistance in the field. Brian Walker for excellent technical assistance in the laboratory. Chantale Côté for helpful comments on an earlier version of the manuscript. Raymond Vezeau for providing access to Environment Canada laboratory facilities. François Gagné for providing unpublished *Mya arenaria* MFO measurements. Deborah Steel of the Institut Maurice Lamontagne in Mont-Joli, Quebec for helping us locate unpublished documents on the Saguenay river. The project was funded by a St. Lawrence Centre - Natural Sciences and Engineering Research Council of Canada research partnership grant to JBR and a Natural Sciences and Engineering Research Council of Canada doctoral fellowship to PAW.

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CHAPTER 8

GENOTOXIC SUBSTANCES IN FISH AND MACROINVERTEBRATES FROM THE ST. LAWRENCE AND SAGUENAY RIVERS (CANADA)

INTRODUCTORY REMARKS

The previous chapter demonstrated the use of the SOS Chromotest for the detection of genotoxic organics in the tissues of soft-shell clams (*Mya arenaria*). In the final chapter of the thesis the SOS Chromotest method developed in Chapter 1, and adapted to biota analyses in Chapter 7, is used to investigate the presence, bioaccumulation and biomagnification of genotoxins in aquatic biota collected at several sites on along the St. Lawrence and Saguenay rivers. With minor exceptions, the sites investigated are the same as those examined in Chapter 5.

The material presented was prepared for submission to *Environmental Toxicology and Chemistry*. Both Prof. Josep¹ Rasmussen and Dr. Christian Blaise played a role in the development of the ideas presented. They are second and third coauthors respectively.

Appendix 10 contains the data used to generate all figures and tables.

ABSTRACT

Aquatic biota frequently accumulate organic contaminants and maintain steady state tissue concentrations that are as much as 10^5 times higher than those in the surrounding water. Although many researchers have studied the accumulation of genotoxic PAHs by aquatic biota, few researchers have used bioassays to investigate the accumulation of genotoxins. In several previous studies we used the SOS Chromotest to investigate the genotoxicity of industrial effluents extracts, sediment extracts and bivalve tissue extracts. In this study we use the SOS Chromotest to investigate the accumulation of organic genotoxins by macroinvertebrates and fish in the St. Lawrence and Saguenay rivers. Tissue concentrations of genotoxins (expressed as μg benzo(a)pyrene genotoxic equivalents) reveal bioconcentration factors in the 10^2 to 10^3 range. Concentrations are partially determined by the lipid content ($r^2 = 0.22$). Lipid normalized values indicate that genotoxin concentrations in invertebrate tissues are significantly higher than those of fish. Fish values indicate that tissue concentrations are bio-diminished, with fish at higher trophic levels having lower tissue burdens of genotoxins. The bio-diminution observed corresponds exceptionally well to the trophic position assignments made by other authors. More contaminated sites yielded less contaminated specimens. This may be due to the induction of phase I and phase II detoxification enzymes that is likely to occur at high levels of exposure. Although the results do not support PAHs as the putative genotoxins, they do indicate that the accumulated genotoxins have similar properties. Tissue to sediment ratios of genotoxins are similar to those observed for genotoxic PAHs, and far lower than those of more persistent organochlorines. Although we did not investigate genotoxic effects, we might expect the most dramatic effects in biota that consume contaminated macroinvertebrates.

INTRODUCTION

Chronic exposure and accumulation of organic contaminants by aquatic biota can result in tissue burdens that have adverse effects on the exposed organisms (e.g. tissue necrosis and hyperplasia, reproductive failure, neoplasia) and/or the consumers of these organisms (Bysshe, 1982). Accumulation occurs when the rate of uptake from the environment exceeds the rate of elimination (depuration). Long-term exposure and contaminant retention can result in steady-state concentrations that are as much as 10^5 times higher than those of the surrounding water (Veith et al., 1979). The tendency for organic contaminants to accumulate is primarily controlled by contaminant solubility, often expressed in terms of the octanol-water partition coefficient (K_{ow} or P_{oc}) (Neely et al., 1974; Mackay, 1982; Kenaga and Goring, 1980). Contaminants with low solubility and high K_{ow} have a strong tendency to partition into the lipid pools of exposed organisms (Thoman, 1989).

Contaminants enter aquatic biota via direct diffusion across the gill-water interface or uptake and assimilation following the consumption of contaminated food (Spacie and Hamelink, 1985). Thus, in addition to lipid solubility and persistence, accumulation is determined by contaminant (bio)availability and the trophic level of the organism under consideration. The precise role of contaminated food in determining steady-state contamination levels of feral biota is a controversial topic (Woodwell et al, 1967; Rasmussen et al., 1990; Oliver and Niimi, 1988; Moriarty, 1988; Bruggeman et al., 1981; Macek et al., 1979). Early studies of DDT residues in aquatic food chains concluded that biomagnification does occur (Woodwell et al, 1967). However, some researchers argue that convincing evidence is lacking and claim that tissue residues of persistent contaminants can be explained solely by direct partitioning across the gill-water interface (Moriarty, 1985; Bruggeman et al., 1981; Macek et al., 1979). Mechanistic models indicate that efficient transfer of contaminants from prey to predator will occur in long-lived, slow growing organisms that can assimilate contaminants more rapidly than they are metabolized and excreted (Spacie and Hamelink, 1985). However, demonstration of food chain effects in feral populations of aquatic biota has proved challenging. Recently, the importance of food chain structure in determining tissue residues of persistent contaminants in pelagic fish has been confirmed by Rasmussen et al. (1990).

Most of the available information concerning the bioaccumulation and biomagnification of aquatic contaminants has been obtained through investigations of specific substances, or groups of substances. However, monitoring for specific contaminants requires *a priori* knowledge of contaminant identity. An alternative approach to the study of toxicant bioaccumulation involves the use of bioassays to detect (geno)toxic substances in complex

tissue extracts. Tissue burdens of (geno)toxicants can be compared to ambient water and/or sediments levels in order to assess bioaccumulation. In addition, tissue burdens of biota from various trophic levels can be used to assess biomagnification. The ultimate hazard of the toxicants investigated will be dependent on potency as well as ecological behaviour. Those substances that are both lipophilic and resistant to degradation will be accumulated, and may be efficiently transferred from prey to predator.

Although a large number of studies have used bioassays to detect genotoxic or mutagenic substances in industrial wastes (see Houk, 1992 for review), sediments (e.g. Langevin et al, 1992; Ho and Quinn, 1993; Marvin et al, 1993; Balch et al., 1995; White et al., 1995a) and surface waters (see Stahl, 1991 for review), relatively few studies have investigated the accumulation of genotoxins in aquatic biota (see White et al., 1995b for review). In addition, published investigations of aquatic biota are primarily restricted to invertebrates (e.g. Rodriguez-Ariza et al., 1992; Marvin et al., 1994) that are renowned for their ability to accumulate genotoxic polycyclic aromatic hydrocarbons (PAHs) such as benzo(a)pyrene (Stegman and Lech, 1985; Dunn, 1991). To our knowledge no published studies have examined biota from several trophic levels in an effort to investigate both bioaccumulation and biomagnification. Genotoxic substances that bioaccumulate and biomagnify could constitute a serious health hazard for both indigenous biota and humans.

In several previous works we demonstrated the use of the SOS Chromotest (Quillardet et al, 1982; White et al., 1995c) to detect complex mixtures of genotoxins in industrial effluents (White et al., 1995d), river sediments (White et al., 1995a) and bivalve molluscs (White et al., 1995b). While our sediment analyses provided some insight into the persistence of genotoxic substances, we did not investigate other ecotoxicological phenomena. In this study we extend our analyses to include macroinvertebrates and fish collected from sites that receive genotoxic industrial and municipal discharges (White et al., 1995d). The results are used to investigate the bioaccumulation and biomagnification of genotoxins in the St. Lawrence and Saguenay system.

MATERIALS AND METHODS

Study Site and Sample Collection-

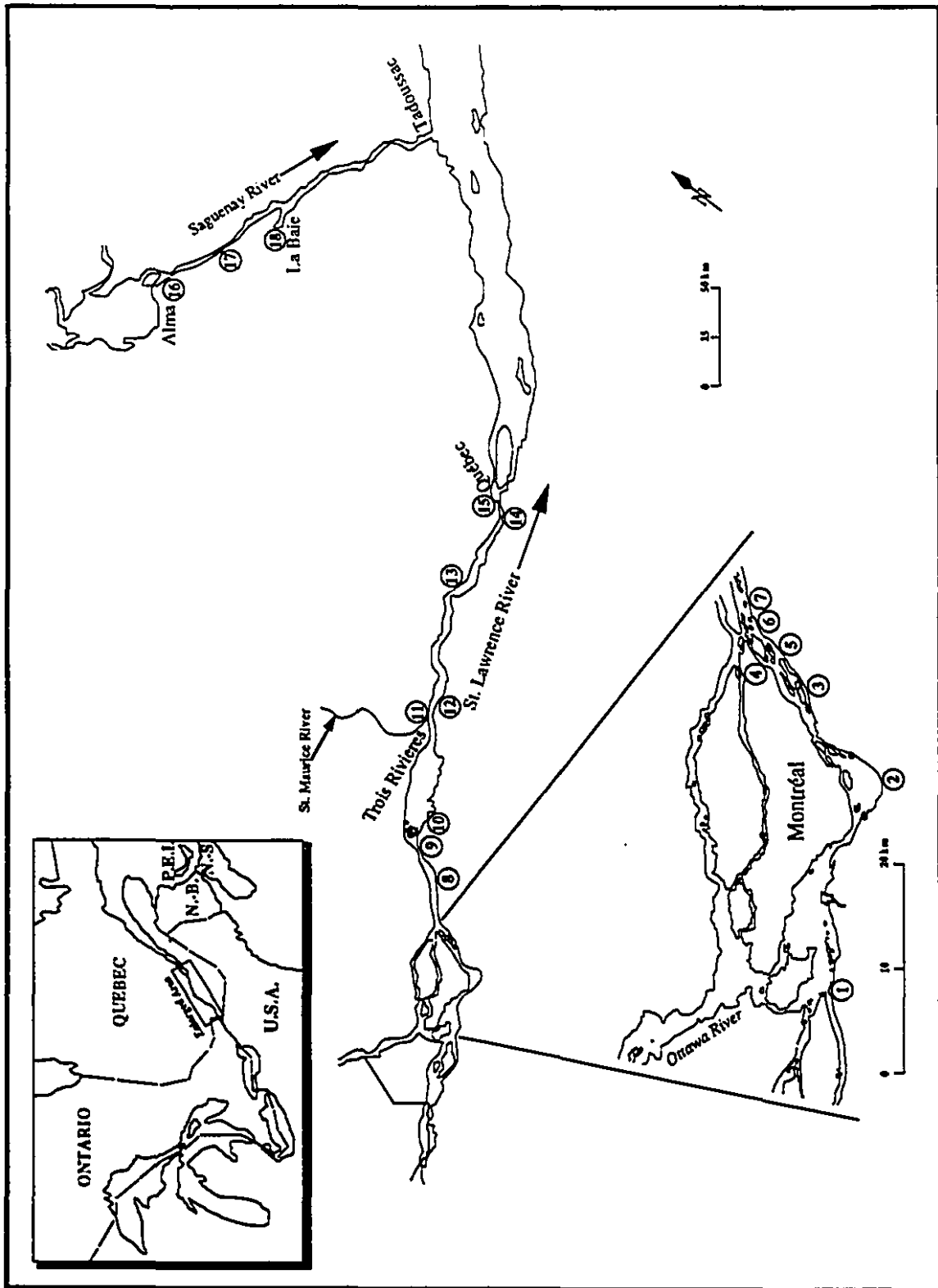
Biota samples were collected from 18 sites along the St. Lawrence and Saguenay rivers. The sites investigated receive industrial and municipal wastes from facilities that have been identified by the government of Québec and the government of Canada as priorities for concern and control (CSL, 1992). Many of the facilities are known to release genotoxic organic contaminants (White et al., 1995d, EIS, 1992). In addition, previous investigations indicate that in many of the areas investigated suspended and sedimented particulate material is contaminated with genotoxic substances. The location of all sampling sites is illustrated in Figure 1. Additional sampling site information can be found in White et al. (1995a). In addition to specimens collected from the St. Lawrence system, samples of fish and macroinvertebrates were also collected from Lake Memphremagog (≈ 100 km southwest of Montreal) and Lake Onatchiway (≈ 350 km NE of Montreal), two lakes that receive no direct industrial input.

All samples were collected during the summers of 1991 and 1992. As outlined in White et al. (1995a) sample collection sites were located 1000-1500 m downstream from the effluent outfalls previously investigated for genotoxicity (White et al. 1995d). Fish were captured using a variety of 40-80 m x 2 m, 2 to 4 panel, experimental gill nets. Mesh size ranged from 1 in. (2.54 cm) to 4 in. (10.16 cm) (Filmar Industries, Inc., Québec, QC). Captured fish were euthanized by cervical dislocation, measured (total length), wrapped in pre-cleaned aluminum foil and frozen immediately on site (propane-powered freezer, Dometic Corp., LaGrange, IN).

At each site an attempt was made to collect a minimum of 3 Northern Pike (*Esox lucius*) specimens and 6 Yellow Perch (*Perca flavescens*) specimens. Since perch over 15 cm are primarily piscivorous (Scott and Crossman, 1973), an attempt was made to collect 3 large perch (over 15 cm) and 3 small perch (less than 15 cm). Where perch and/or pike were not available, the following species were collected: Walleye (*Stizostedion vitreum*) at Beauport, Donnacona and St. Romuald, Sauger (*Stizostedion canadense*) at Pointe-aux-Trembles, Brook Trout (*Salvelinus fontinalis*) at Jonquière, Atlantic Herring (*Clupea harengus*) and rainbow smelt (*Osmerus mordax*) at La Baie. At Lake Memphremagog specimens of perch (7) and Chain Pickerel (3) (*Esox niger*) were collected. Three pike specimens were obtained from Lake Onatchiway.

Macroinvertebrates were sampled with an 18 x 8 in. (45.7 x 20.3 cm) rectangular dip/kick net with an 800 x 900 μ m mesh (Wildco Wildlife Supply Co., Saginaw, MI). At one site (Beauharnois), benthic invertebrates were also collected with a custom-made

Figure 1. Location of sampling sites. Fifteen sites (sites 1-15) are located on the St. Lawrence river between Beauharnois and Beauport. Three sites (sites 16-18) are located along the Saguenay system between Alma and La Baie. Site names used on subsequent figures are: 1=Beauharnois, 2=La Prairie, 3=Longueuil, 4=Montréal-East, 5=Point-aux-Trembles, 6=Boucherville, 7=Varennnes, 8=Contrecoeur, 9=Tracy, 10=Sorel, 11=Trois Rivières, 12=Bécancour, 13=Donnacoona, 14=St. Romuald, 15=Beauport, 16=Alma, 17=Jonquière, 18=La Baie.



benthic sled. Organisms collected include a wide variety of gastropods, insect nymphs and larvae, crustaceans and oligochaetes. For this preliminary investigation of genotoxins in aquatic biota, little attempt was made to separate the samples into their respective taxonomic groups. Samples were collected such that the total wet weight exceeded a minimum of 10 g. Where quantities permitted, samples were separated into gastropods, amphipods (*Gammarus spp.*), decapods (primarily *Orconectes spp.*) and miscellaneous riparian invertebrates. Gastropod samples consisted primarily of Bithyniidae, Planorbidae and Physidae. Mixed invertebrate samples contained mostly hemipterans (e.g. Corixidae and Notonectidae), odonates (both Zygoptera and Anisoptera) and oligochaetes. Samples were separated from debris, placed in solvent-washed glass jars and frozen immediately on site. Samples collected from Lake Memphremagog contained primarily insect nymphs belonging to the orders Trichoptera and Odonata (primarily Anisoptera).

Preparation of Biota Extracts-

Specimens less than 50 g wet weight were extracted whole. Small fish were combined to provide composite samples. For larger individuals, extracts were prepared from 50 g samples of epaxial muscle taken from the caudal region. Biota extracts were prepared as described in White et al. (1995b). Briefly, wet samples (= 35-50 g) were blended with 400-500 mL of DCM (dichloromethane, CAS # 75-09-2, Anachemia Science, Montreal) in stainless steel cups using a high-speed (3500 rpm) industrial blender (Eberbach Corp., Ann Arbor, MI). Each sample was blended for 3 x 4 minutes and subsequently sonicated (ultrasonic cell disrupter- Branson Sonic Power Co., Danbury, CT) on ice for 2 x 3 minutes. Blending and sonication periods were alternated with 3-4 minute cooling periods. Solid material was removed via filtration through a 2 cm layer of Kieselguhr (CAS # 61790-53-2, diatomaceous earth - A&C American Chemicals, Montreal) overlaying a coarse sintered glass. Extracts were dried with anhydrous sodium sulfate (CAS # 7757-82-6, Mallinckrodt Specialty Chemicals, Mississauga, Ontario) and reduced to approximately 5 mL by rotary evaporation at 30°C (Büchi Laboratorium-Technik AG, Flawil/Schweiz, Switzerland). All extracts were taken to dryness under a gentle stream of ultra-pure N₂ gas. Prior to resuspension, the mass of the extractable residue was determined gravimetrically. The weight of this residue was used as an estimate of lipid content (Engel, 1985). Extracts were resuspended in dichloromethane in preparation for gel permeation clean-up.

As described in White et al. (1995a) and White et al. (1995b) the extraction method has been validated by spiking samples of sediment, suspended particulate matter and freshwater clams (Unionidae) with a mixture of 15 polycyclic aromatic hydrocarbons (Supelco PAH

Mix 4-8905, Supelco Canada Inc., Oakville, Ontario). For this study a sample of fish (chain pickerel - *Esox niger*) muscle tissue was spiked with the same PAH mixture. Mean recoveries of 3-, 4- and 5-ring PAHs were 64%, 68% and 89% respectively.

All labware were precleaned with pesticide grade acetone and hexane (Anachemia Science, Montreal). Glassware were baked at 350°C to remove solvent traces and covered with pre-cleaned aluminum foil. Sodium sulfate and Kieselguhr were combusted at 450°C before use.

Gel Permeation Chromatography

Lipids removal was accomplished using GPC (gel permeation chromatography) on Bio-Beads™ SX-3 (Bio-Rad Laboratories, Mississauga, Ontario). The method employed is a variation on EPA method 3640 (USEPA, 1986) incorporating some of the modifications described by Czuczwa and Alford-Stevens (1989) and Engel (1985). The method is fully described in White et al. (1995b). Briefly, ≈ 50 g of Bio-Bead™ resin was permitted to swell overnight in 45% dichloromethane in hexane (Anachemia Science, Montreal) and packed into 35 cm (≈175 mL) glass columns (Corning, Richmond Hill, Ontario). Biotra extracts equivalent to approximately 250 mg lipid were applied to pre-calibrated columns and eluted under gravity at 1 mL min⁻¹. The initial fraction (80-90 mL) was collected for lipid content determination. Sample elution continued to a total elution volume of 250 mL. Eluted samples were reduced to approximately 5 mL by rotary evaporation at 30°C and taken to dryness under vacuum at low temperature (Speedvac AS290 Concentrator, Savant Ltd., Farmingdale, NY). The weight of the post-GPC residue was measured and the remaining extract dissolved in high-purity DMSO (dimethyl sulfoxide) (Sigma Chemical Co., St. Louis, MO). An aliquot of the post-GPC residue equivalent to ≈1 g of wet tissue was retained for PAH (polynuclear aromatic hydrocarbon) analyses.

GPC columns were calibrated with a dichloromethane solution containing 100 mg mL⁻¹ corn oil, 2 mg mL⁻¹ pentachlorophenol (CAS # 87-86-5, Aldrich Chemical Co., Milwaukee, WI), 2 mg mL⁻¹ bis(2-ethylhexyl) phthalate (CAS # 117-81-7, Aldrich Chemical Co., Milwaukee, WI), 0.04 mg mL⁻¹ perylene (CAS # 198-55-0, Aldrich Chemical Co., Milwaukee, WI) and 0.1 mg mL⁻¹ sulfur (CAS # 7704-34-9, Anachemia Science, Montreal). Flow conditions (under gravity) and lipid load were adjusted to optimize the separation of corn oil lipids from bis(2-ethylhexyl) phthalate. Elution of calibration compounds was monitored by absorbance at 254 nm (Beckman DU-70 UV-VIS spectrophotometer, Beckman Instruments, Palo Alto, CA). Dump volumes were adjusted to permit >85% removal of the lipids and >85% recovery of the bis(2-ethylhexyl) phthalate

(USEPA, 1986). Dump volumes ranged from 80-90 mL with complete sulfur elution at approximately 160 mL.

SOS Chromotest Analyses

Genotoxicity of tissue extracts was measured using the SOS Chromotest (Quillardet et al., 1982; Quillardet and Hofnung, 1985). The test involves incubation of *Escherichia coli* PQ37 (*lacΔU169 uvrA rfa sulA::Mud(Ap.lac) cts PhoC*) with the pure compound or complex mixture to be investigated. The *sulA::Mud(Ap.lac)* fusion places the production of functional β -galactosidase under the express control of the SOS response pathway which is induced by DNA damaging agents (Walker, 1984). Post-exposure β -galactosidase activity reflects the genotoxicity of a given sample. Alkaline phosphatase activity provides an indirect measure of cell viability (Quillardet et al., 1982). Initial genotoxicity analyses conducted in the presence of a post mitochondrial supernatant (S9 fraction) obtained from Arochlor 1254 induced rat liver (Molecular Toxicology Products Inc., Annapolis, MD) indicated that S9 enzymes reduced sample potency. In the present work, only the direct acting (without S9) results will be discussed. 4-Nitroquinoline-1-oxide (CAS # 56-57-5, Sigma Chemical, St. Louis, MO) was used as a positive control.

The semi-automated SOS Chromotest protocol employed is described in detail in White et al. (1995c). Briefly, a 16 hour culture (37°C) of *E. coli* PQ37 was diluted in fresh medium (Luria-Bertani (LB) broth + 20 μ g per mL ampicillin) to an optical density (600 nm) of 0.05-0.075. The sample (or solvent blank), dissolved in 10 μ L DMSO was added to 190 μ L of diluted culture (plus S9 if desired) present in the first well of each dilution series and serially diluted to provide a total of six, 2-fold dilutions. Maximum tested concentrations were $\approx$ 2-30 g wet tissue per assay mL. Twelve control wells received PQ37 culture and blank solvent. Each extract was tested in duplicate at the highest possible concentration range. The final background concentration of DMSO was 5% v/v.

Microplates were incubated for 3 hours at 37°C and subsequently centrifuged at 2000 g for 20 minutes. The supernatant was removed and the bacterial pellet resuspended in 100 μ L of 200 mM (Tris[hydroxymethyl])-aminomethane (CAS # 77-86-1) pH 7.5 and 100 μ L of cell lysis/enzyme assay buffer (40% v/v methanol (CAS # 67-56-1), 0.5% v/v toluene (CAS # 108-88-3), 10% v/v N,N-dimethyl formamide (68-12-2), 4 mg/mL 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-Gal, CAS # 7240-90-6)), 1 mg/mL *p*-nitrophenyl phosphate (CAS # 4262-83-9)). Plates were mixed for 60 seconds (Titertek microplate vortex, Flow Laboratories, Lugano, Switzerland) and incubated (37°C) for 60 minutes after which *initial* optical density readings (Titertek Multiskan MCC/340 Microplate

Spectrophotometer) were taken (620 nm and 405 nm). Final optical density readings were taken 120 minutes after cell resuspension and lysis. Optical density values recorded at 620 nm reflect the activity of β -galactosidase, those recorded at 405 nm measure the activity of alkaline phosphatase. Calculation of net optical density values (120 min value - 60 min value) allowed the contribution of any remaining colour to be accounted for prior to data analysis.

Data Analyses

Induction of the SOS response pathway at a sample concentration C , denoted $R(C)$, is the ratio of net (120 min - 60 min) β -galactosidase activity to net alkaline phosphatase activity. To account for the background induction of the *sulA* gene, a normalized SOS response induction factor was calculated as $IF = R(C)/R(0)$, where $R(0)$ is the ratio of β -galactosidase to alkaline phosphatase activity averaged over the 12 controls which received blank solvent. A normalized induction factor that exceeded the upper 95% confidence limit of the control was regarded as a significant positive. Since normalized induction factor values are ratios of two means, confidence limits were calculated according to Welsch et al. (1988). Concentration response curves of normalized induction factor (IF) against sample concentration (equivalent g of wet tissue per assay mL) were inspected and the results qualitatively categorized as follows-

- 1) Negative- induction factor never exceeded the upper confidence limit of the control;
- 2) Marginal- induction factor exceeded the upper confidence limit of the control at one or two concentrations only;
- 3) Positive- induction factor exceeded the upper confidence limit of the control at a minimum of three doses;
- 4) Erratic- induction factor may have exceeded the control at several concentrations, but the concentration-response relationship was erratic and could not be interpreted.

Genotoxic potency values were calculated for each sample that elicited positive or marginal responses. The SOS response inducing potency (SRIP), expressed as SOS Induction Factor units per equivalent g of wet tissue, is the initial slope of the concentration response curve. The minimum detectable genotoxic concentration (MDGC) is the concentration of extract (equivalent g wet tissue per assay mL), inferred from the fitted concentration-response curve, required to produce an induction factor that equals the upper

95% confidence limit of the control. The maximum level of SOS induction (MaxIF) was also noted and recorded.

When concentration response curves were distinctly nonlinear, exhibiting zero-order kinetics at high sample concentration, they were analyzed by iterative, non-linear regression employing a least squares loss function (SAS Institute, 1988). In these cases, the SRIP was calculated according to Langevin et al. (1992). When the range of concentrations tested did not result in a full hyperbola, the SRIP was determined by ordinary, least squares linear regression.

All data analyses were performed using the SAS system version 6.10 for OS/2™ (SAS Institute, 1993). Analysis of covariance (ANCOVA), analysis of variance (ANOVA) and least squares linear regression were used to investigate patterns in the results. Where necessary the data were log transformed to equalize the variance across the range of observations and meet the assumptions of linear regression, ANOVA and ANCOVA (Ricker, 1973). The residual error associated with all regression, ANOVA and ANCOVA models were assumed to be independent and normally distributed. Normality was assessed using the Shapiro-Wilk test and visual examination of a normal probability plot (SAS Institute, 1988; Zar, 1984).

Polycyclic Aromatic Hydrocarbon (PAH) Analyses

PAH analyses were conducted for 88 biota extracts (68 fish samples and 20 invertebrate samples). For each sample analyzed, an aliquot of the post-GPC material equivalent to ≈ 1 g of wet tissue was dissolved in 100 μ L pesticide-grade benzene (Anachemia Science, Montreal). Samples were spiked with d_{10} -fluoranthene (Supelco Canada, Oakville, Ontario), the internal standard. PAH concentrations were measured via gas chromatography (GC)/mass spectrometry (MS) (HP 5890 Series II GC with HP 5971A MS, Hewlett-Packard Canada, Ltd., Mississauga, Ontario) in SIM (selected ion monitoring) mode. Detection limits were $\approx 0.5 - 1.0$ ppb (ng per g wet wt.). A list of the PAHs analyzed is provided in Table 1. 1 μ L of each sample was injected *on column* with the following GC/MS conditions:

Column- J&W DB-5 30 m x 0.25 mm (Alltech Associates Inc., Guelph, Ontario);

Mass Range- 127-302 amu;

Temperature program- 80°C for 2 min, 80°-230°C at 10°C per min,

230°-300°C at 20°C min⁻¹;

Carrier gas- He at 8 psi, 80°C with constant flow.

The Following abbreviations are used throughout the text-

MaxIF = maximum SOS induction factor observed. BaP = benzo(a)pyrene. PAH = polycyclic aromatic hydrocarbons. ppb = parts per billion or ng per g. ppm = parts per million or μg per g.

Table 1. Polycyclic aromatic hydrocarbons investigated in this study. All genotoxic PAHs require S9 metabolic activation. Genotoxic equivalency factors (GEF) reflect the potency of each PAH relative to BaP. In an identical manner to that done in White et al. (1995a), GEF values were used to convert PAH measurements to their BaP equivalence. In concept, the GEF values used here are analogous to the toxic equivalency factors (TEF) used to calculate concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxic equivalents in fish and bird tissues (van den Heuval, 1994; Tillitt et al., 1991; Ankley et al., 1991).

Compound	CAS #*	Molec. Wt (g/mole)	Genotoxic?/ Carcinogenic?‡	GEF¶
Naphthalene (NAPH)	91-20-3	128.2	0/0	0.00
Acenaphthylene (ACENY)	208-96-8	152.2	NA	0.00
Acenaphthene (ACEN)	83-32-9	154.2	0/NA	0.00
Fluorene (FLU)	86-73-7	166.2	0/I	0.00
Phenanthrene (PHEN)	85-01-8	178.2	++?/I	0.101
Anthracene (ANTH)	191-26-4	178.2	+/0	0.0330
Fluoranthene (FLUOR)	206-44-0	202.3	++/0	0.398
Pyrene (PYR)	129-00-0	202.2	++?/0	0.0534
Benzo(c)phenanthrene (B(c)P)	195-19-7	228.3	+/I	1.50
Benz(a)anthracene (B(a)A)	56-55-3	228.3	++/++	0.0211
Chrysene (CHRY)	218-01-9	228.3	++?/+	0.104
Benzo(j)fluoranthene (B(bjk)F)	205-82-3	252.3	+/++	0.262
Benzo(b)fluoranthene	205-99-2	252.3	++?/++	--
Benzo(k)fluoranthene	207-08-9	252.3	+/++	--
Benzo(a)pyrene (B(a)P)	50-32-8	252.3	+++ / ++	1.000
Indeno(1,2,3- <i>cd</i>)pyrene (I(123cd)P)	193-39-5	276.3	+ / ++	0.0283

Table 1 continued.....

Dibenz(<i>a,h</i>)anthracene (DB(ah)A)	53-70-3	278.3	++/++	0.0391
7,12-Dimethylbenz(<i>a</i>)anthracene (DMBA)	39834-38-3	256.3	+ / ++	0.208
3-Methylcholanthrene (3MC)	56-49-5	268.3	+ / ++	0.215
Benzo(<i>ghi</i>)perylene (B(<i>ghi</i>)P)	191-24-2	276.3	+ / I	0.0249
Dibenz(<i>a,h</i>)pyrene (DB(ah)P)	189-64-0	302.4	+ ? / ++	0.0381
Dibenz(<i>a,l</i>)pyrene (DB(al)P)	191-30-0	302.4	+ / ++	1.19
Dibenz(<i>a,i</i>)pyrene (DB(ai)P)	189-55-9	302.4	+ / ++	0.228

*Chemical Abstracts Service registry number.

†Based on information from IARC (1983); Hermann (1981); Sakai et al. (1985); LaVoie et al. (1980); Mersch-Sundermann et al. (1992); Mersch-Sundermann et al. (1993); Gold et al. (1984); Gold et al. (1986). Genotoxicity symbols are: 0 - no evidence of genotoxicity, + - indicates positive response on prokaryotic short-term tests, ++ - indicates positive response on both prokaryotic and eukaryotic short-term tests, ? - indicates conflicting results reported in the literature. Carcinogenicity symbols are: 0 - no evidence of carcinogenicity, I - inadequate evidence for evaluation, + - limited evidence of carcinogenicity in experimental animals, ++ - sufficient evidence of carcinogenicity in experimental animals. NA indicates data not available.

‡Data are from measurements made in our laboratory as well as Mersch-Sundermann et al. (1992), Mersch-Sundermann et al. (1993), Mersch-Sundermann et al. (1994), Ohta et al. (1984), Von der Hude et al. (1988) and Quillardet et al. (1985).

§Results reported as the sum of benzo(*b*)fluoranthene, benzo(*k*)fluoranthene and benzo(*j*)fluoranthene.

RESULTS

A total of 152 biota samples (120 fish samples, 32 invertebrates) were analyzed for genotoxicity. In the absence of metabolic activation roughly 70% of the fish extracts yielded a positive, marginal or erratic response. Roughly 88% of the invertebrate samples yielded a positive, marginal or erratic response. Figure 2 illustrates concentration-response relationships for several positive samples from St. Romuald, Contrecoeur and Donnacona. Of the 13 samples (9 fish, 4 invertebrate) collected from Lake Memphremagog, 2 perch extracts yielded marginal, positive responses. Both chain pickerel samples failed to elicit a positive response. In addition, none of the four invertebrate samples elicited a positive response. In the absence of a positive response from the invertebrate extracts, the two weak perch responses are suspect. None of the three pike samples from Lake Onatchiway elicited a positive response.

Where concentration-response relationships are non-linear and approach zero-order kinetics at high concentrations, the maximum SOS Induction Factor reached reflects the point at which the response pathway of the bioassay is saturated. It was immediately apparent that the majority of the samples examined failed to elicit maximum SOS Induction Factors above 2.0. Only two extracts elicited Induction Factors above 2.5. This is in contrast to the results obtained in our previous analyses of *Mya arenaria* from the Saguenay Fjord. *Mya arenaria* extracts frequently elicited MaxIF values above 3.0. In several cases MaxIF values approached 5.0. Figure 3 summarizes the mean MaxIF values for the various biota samples investigated in this study and White et al. (1995b). ANOVA revealed a significant effect of biota type (3 categories- fish, invertebrates, *Mya*) on mean MaxIF. Post-hoc mean comparisons revealed that the mean fish MaxIF and the mean invertebrate MaxIF are both significantly different from the mean *Mya* MaxIF.

Since genotoxins in environmental extracts are chemically diverse, it is useful to express the results as equivalents of a standard reference compound. All genotoxicity values were thus converted to their BaP (benzo(a)pyrene) genotoxic equivalence and expressed as μg BaP Equivalents per g wet tissue. Tissue concentrations of BaP equivalents were calculated as the ratio of the MDGC of benzo(a)pyrene to the MDGC of the sample investigated. Minimum, maximum and mean tissue concentrations of BaP equivalents are summarized in Table 2. The results indicate that small perch (≤ 15 cm) are the most contaminated samples, followed by the invertebrates, larger perch and finally pike and walleye samples.

Comparisons of tissue concentrations summarized in Table 2 may not be valid. Organic contaminants in water have a tendency to partition into the lipid pools of the

Figure 2. SOS Chromotest concentration response plots for dichloromethane extracts of biota collected from 3 sites on the St. Lawrence river. Mean SOS Induction factors values are based on concentrations tested in duplicate. Error bars are one standard error of the mean. Where error bars are not shown they were smaller than the plotting symbol. All results shown were obtained in the absence of S9 metabolic activation. The upper 95% confidence limit of the solvent control was calculated according to the method of Welsch et al. (1988).

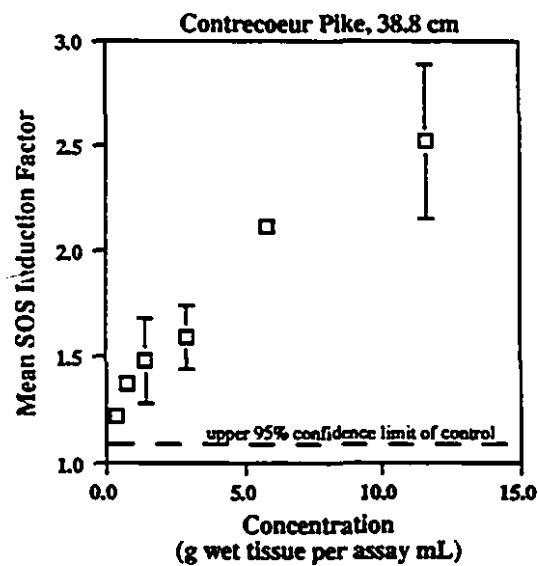
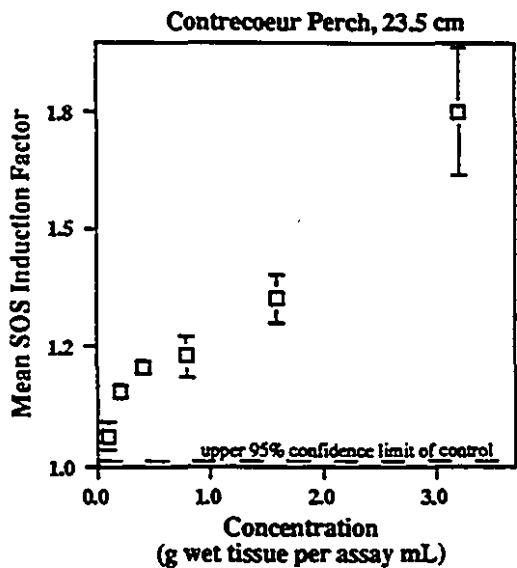
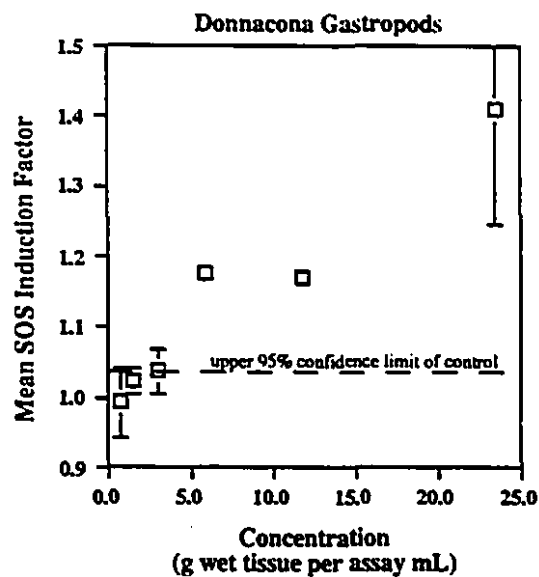
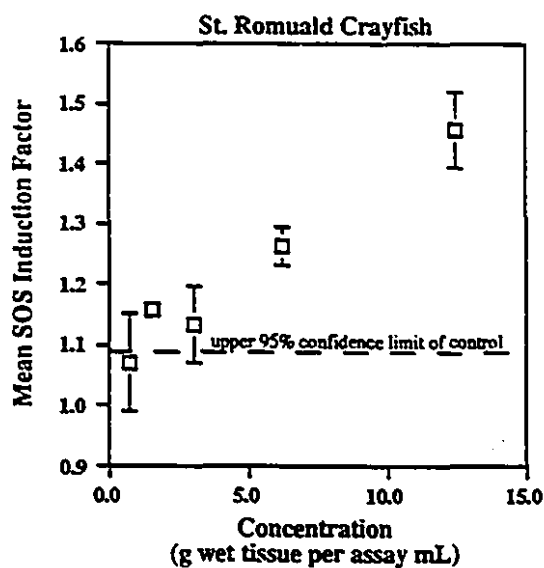
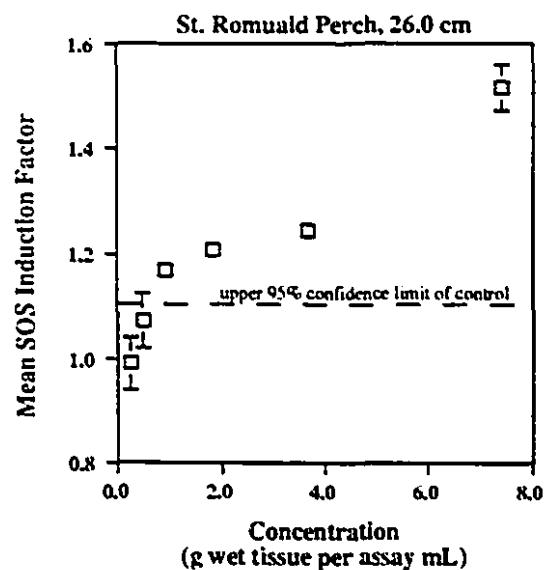
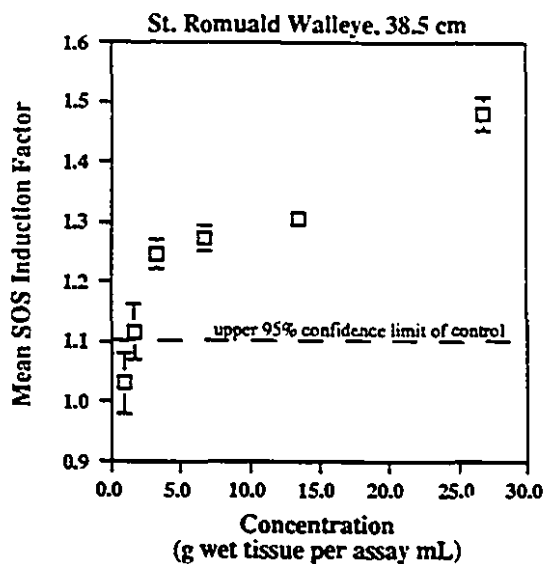


Figure 3. Mean MaxIF values for various biota tissue extracts. Values shown are arithmetic means. Error bars are one standard error of the mean. Where error bars are not shown they were too small to be shown. *Mya arenaria* data are from White et al. (1995b). Letters that accompany the first three bars indicate the results of post-hoc contrasts. Bars accompanied by the same letter are not significantly different at $\alpha=0.05$.

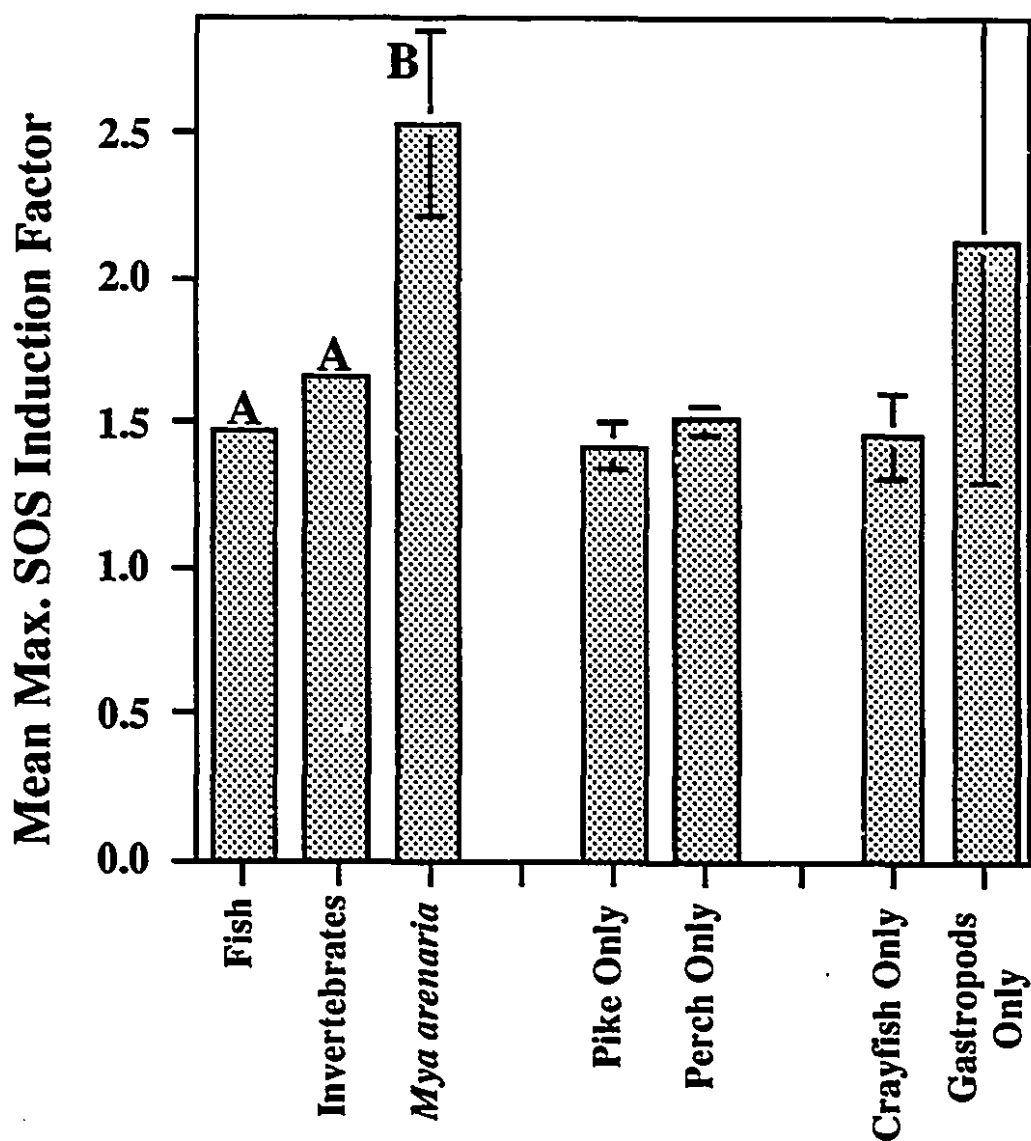


Table 2. Minimum, maximum and mean tissue genotoxicity levels. All values are expressed in μg BaP equivalents per g wet tissue. Large perch are those > 15 cm. Small perch are ≤ 15 cm.

Sample	Min	Max	Mean	Std. Error	N
All Fish	0.0014	10.3	0.58	0.14	94
All Perch	0.0014	10.3	0.73	0.21	58
Perch > 15 cm Only	0.0014	1.31	0.27	0.052	38
Perch ≤ 15 cm	0.028	10.3	1.6	0.55	20
Pike Only	0.0030	0.591	0.15	0.043	20
Walleye Only	0.0018	0.176	0.033	0.024	7
All macroinvertebrates	0.0055	1.7	0.22	0.084	22
Crayfish only	0.012	1.7	0.35	0.26	6
Gastropods	0.0068	0.52	0.086	0.055	9
Mixed Littoral [†]	0.093	0.79	0.43	0.21	3

[†] Samples contained a mixture of oligochaetes, insect nymphs and amphipods.

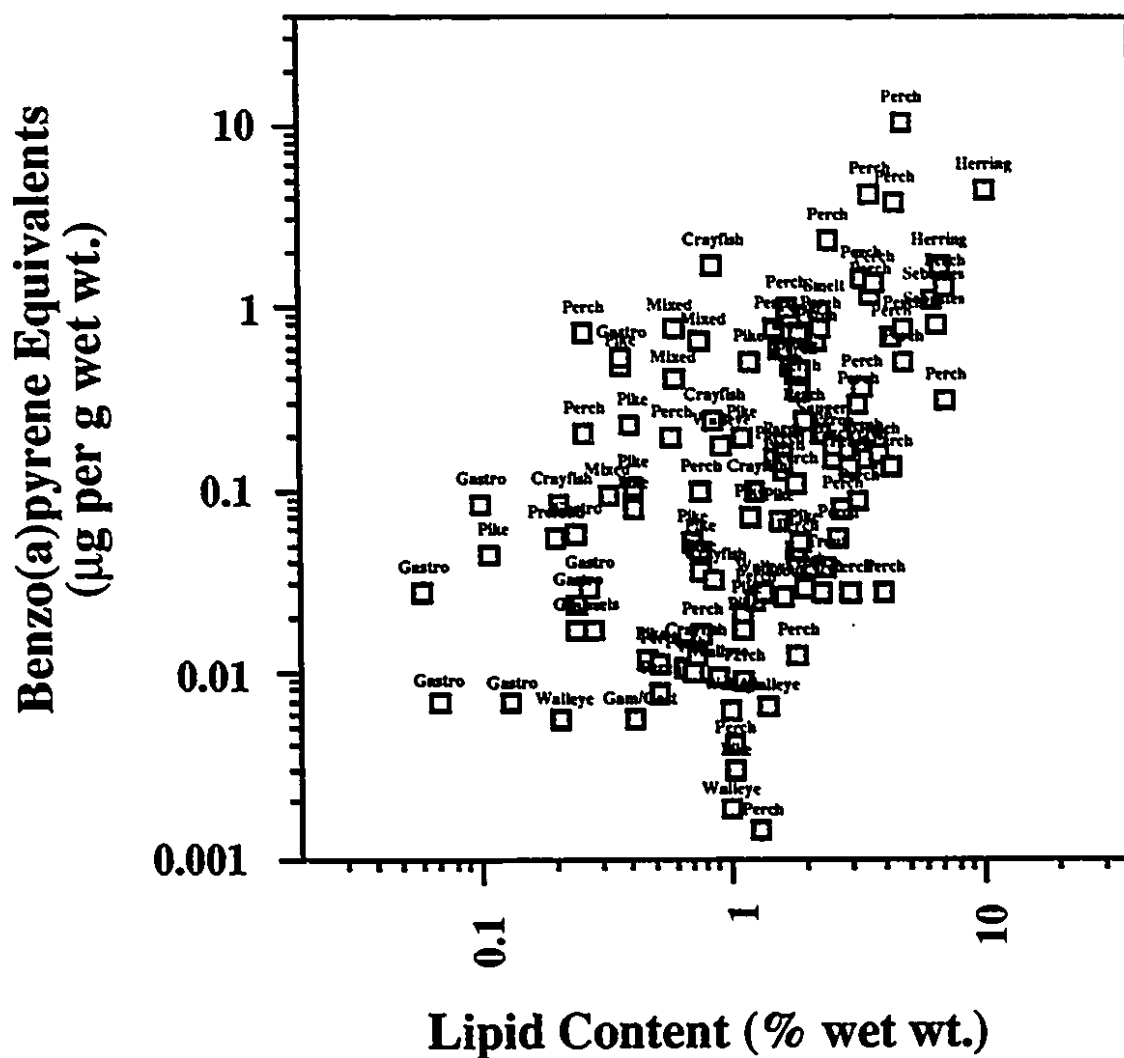
exposed organisms (Spacie and Hamelink, 1985). As a result concentrations are affected by the lipid content of the exposed organism. We have no reason to assume that genotoxic organics would not behave in a similar manner. Lipid contents of the biota samples examined in this study vary from a low of <1% (wet weight) for macroinvertebrates to a high of over 8% for herring from La Baie. Mean lipid content values for the samples examined are summarized below in Table 3.

Table 3. Measured lipid concentrations for aquatic biota investigated in this study.

Sample	Mean Lipid Content (% wet wt.)	Std. Error
All Fish	2.26	0.18
All invertebrates	0.48	0.071
Perch	2.58	0.20
Pike	0.94	0.12
Trout	2.00	0.23
Walleye	1.17	0.17
Herring	8.75	1.85
Crayfish	0.86	0.11
Gastropods	0.19	0.035
Mixed Littoral Inverts.	0.51	0.090

Although Table 2 indicates that perch are more contaminated with genotoxins than invertebrates, Table 3 indicates that invertebrates have a much lower lipid content. Figure 4 illustrates the relationship between tissue concentrations of BaP equivalents and lipid content. The results confirm that lipid content does have an effect on genotoxic contamination. In addition, for a given lipid content, macroinvertebrates samples are frequently more contaminated than fish. Analysis of covariance confirmed a significant effect of both lipid content and biota type on the tissue concentration of BaP equivalents. The following model relates log tissue concentration of BaP equivalents ($\mu\text{g per g}$) to log

Figure 4. The effect of lipid content (% wet wt.) on tissue concentrations of genotoxins (as μg BaP equivalents per g wet tissue). Value labels indicate the type of biota sampled. Gastro=gastropods, Gam/Gast= *Gammarus*/gastropods, Mixed= mixed invertebrates, Profundal= mixed benthic sled sample.



lipid content (% wet wt.) and biota type (fish/invert). ($r^2 = 0.28$, $n = 116$, F Ratio = 21.5, $p < 0.0001$):

$$\text{Log BaP Equivalents} = \begin{bmatrix} -1.15 \text{ All Fish} & \text{A} \\ -0.65 \text{ All Inverts} & \text{B} \end{bmatrix} + [1.12 \pm 0.18 * \text{Log Lipid (\% wet wt.)}] \quad (1)$$

($\mu\text{g per g wet tissue}$)

All effects are significant at $p < 0.02$. The value associated with the slope term is the standard error of the estimate. Post-hoc contrasts of regression coefficients (SAS Institute, 1987) revealed a significant difference between invertebrate contamination and fish contamination. Coefficients accompanied by the same letter are not significantly different at $\alpha = 0.05$. This result indicates that predicted invertebrate levels will be about half an order of magnitude higher than fish levels. Further analyses of the fish data revealed that for a given lipid content the more piscivorous species generally have lower tissue levels of genotoxins. ANCOVA revealed the following model for fish data only ($r^2 = 0.35$, $n = 85$, F Ratio = 10.7, $p < 0.0001$):

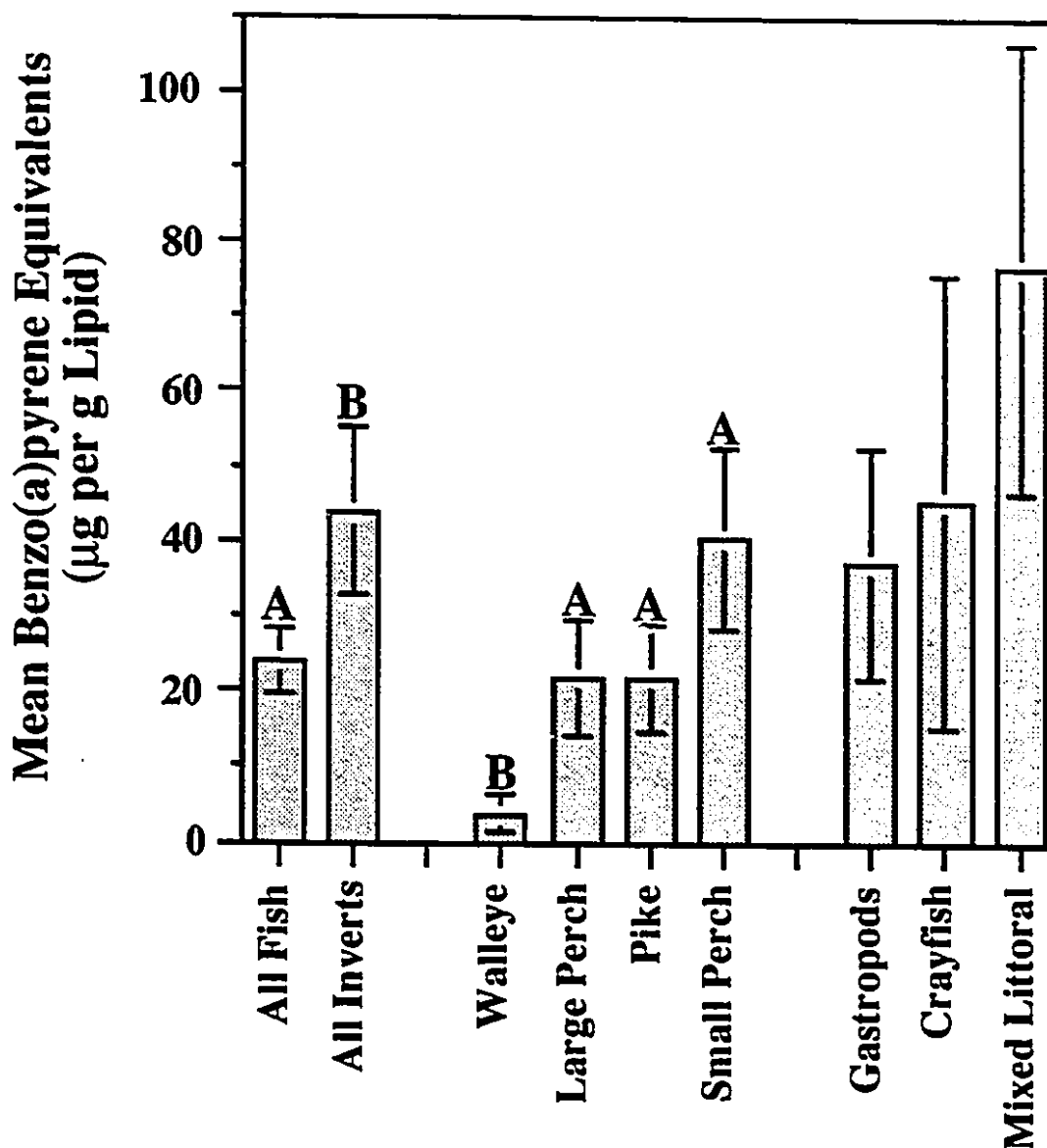
$$\text{Log BaP Equivalents} = \begin{bmatrix} -0.58 \text{ Small Perch} & \text{A} \\ -1.12 \text{ Large Perch} & \text{B} \\ -1.12 \text{ Pike} & \text{B} \\ -1.91 \text{ Walleye} & \text{C} \end{bmatrix} + [0.61 \pm 0.27 * \text{Log Lipid (\% wet wt.)}] \quad (2)$$

($\mu\text{g per g wet tissue}$)

All effects are significant at $p < 0.03$. The value associated with the slope term is the standard error of the estimate. Again, Coefficients accompanied by the same letter are not significantly different at $\alpha = 0.05$. Thus, for a given lipid content small perch have significantly higher tissue levels of BaP equivalents than pike/large perch, which in turn are higher than walleye.

When available evidence indicates that contaminants partition into the lipid pools of exposed biota, it is conventional that chemical concentrations be expressed on a per unit lipid basis (Thoman, 1989). Both models (1) and (2) indicate that the coefficient of log lipid content is not significantly different from 1.0. Therefore, the lipid effect is strong enough to justify lipid-correction of genotoxicity values. Figure 5 compares the mean lipid normalized concentrations of genotoxins for the different biota samples investigated. In contrast to the results summarized in Table 2, the results indicate that after correction for differences in lipid content, macroinvertebrate samples are more contaminated than fish. ANOVA comparing only the mean fish value to the mean invertebrate value revealed a

Figure 5. Comparison of mean lipid normalized tissue concentrations of genotoxic substances. Values are arithmetic means. Error bars are one standard error of the mean. Letters adjacent to bars indicate the results of post-hoc contrasts. Separate ANOVAs were performed for each group of bars. Bars accompanied by the same letter are not significantly different at $\alpha=0.05$.



significant difference (F Ratio = 6.1, $p < 0.02$). A separate ANOVA revealed a significant effect of fish-type on mean genotoxic contamination.

The results in Figures 3 and 4 do not address the fact that previous industrial effluent analyses (White et al., 1995d) indicated that genotoxic loadings at the sites examined vary from less than 1 g BaP equivalent per day at La Prairie to over 30 kg per day at Pointe-aux-Trembles. We investigated the effect of total regional loading on the contamination of biota samples collected in the receiving system. The results obtained are illustrated in Figure 6. Regression analyses failed to demonstrate any significant relationship between biota contamination and regional industrial loading. Although there is no significant trend, the results suggest a negative effect of genotoxic loading on genotoxic contamination of downstream aquatic biota.

Despite the lack of relationship between biota contamination and regional genotoxic loading, the biota results are consistent. Sites with contaminated invertebrates tend to have contaminated fish and *vice versa*. Figure 7 illustrates the relationship between fish contamination and invertebrate contamination across the 12 sites for which data are available. Linear regression analyses revealed the following model ($r^2 = 0.34$, F Ratio = 5.1, $p < 0.05$):

$$\begin{array}{l} \text{Log Fish Contamination} = 0.18 + (0.57 * \text{Log Invertebrate Contamination}) \quad (3) \\ (\mu\text{g BaP Equiv per g Lipid}) \quad (\mu\text{g BaP Equiv per g Lipid}) \end{array}$$

Similar to the results shown in Figure 4 and equation (1), the mean concentration of BaP equivalents in fish from a given site are usually, though not always, lower than that observed for invertebrates. In most cases, the mean fish contamination is about half an order of magnitude lower than that of invertebrate samples. It is interesting to note that several of the sites with the lowest genotoxic loading contained the most contaminated biota samples.

The results further indicate that the mean level of genotoxins in piscivorous fish (walleye/pike) from a given site are empirically related to levels in omnivorous species such as perch. Figure 8 illustrates the relationship between mean pike/walleye contamination and perch contamination. Linear regression analysis revealed the following model ($r^2 = 0.64$, F Ratio = 14.2, $p < 0.006$):

$$\begin{array}{l} \text{Log Pike/Walleye Contam} = 0.24 + (0.65 * \text{Log Perch Contamination}) \quad (4) \\ (\mu\text{g BaP Equiv per g Lipid}) \quad (\mu\text{g BaP Equiv per g Lipid}) \end{array}$$

Figure 6. The effect of total regional genotoxic loading from industrial and municipal sources on the observed genotoxic contamination of aquatic biota the St. Lawrence and Saguenay rivers. Loading values are from White et al. (1995d). Where several observations were available biota contamination values are arithmetic means. Number of observations ranged from 1 to 9.

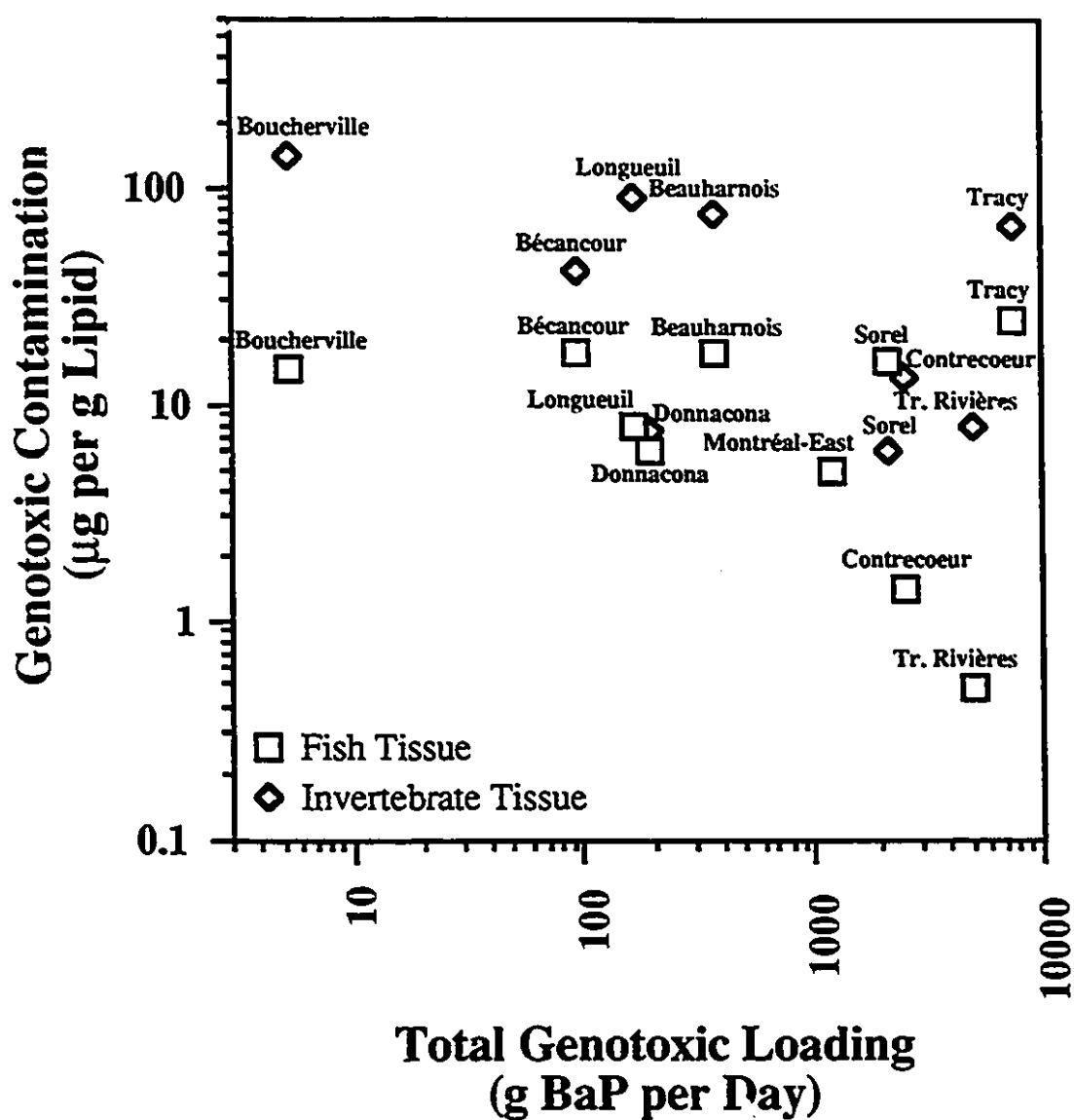


Figure 7. The relationship between the genotoxic contamination of fish and the genotoxic contamination of macroinvertebrates for 12 sites along the St. Lawrence river. Where several observations were available, values are arithmetic means. Number of observations ranged from 2 to 9. Linear regression analysis revealed a significant relationship (see text). The dashed line is the 1:1 line.

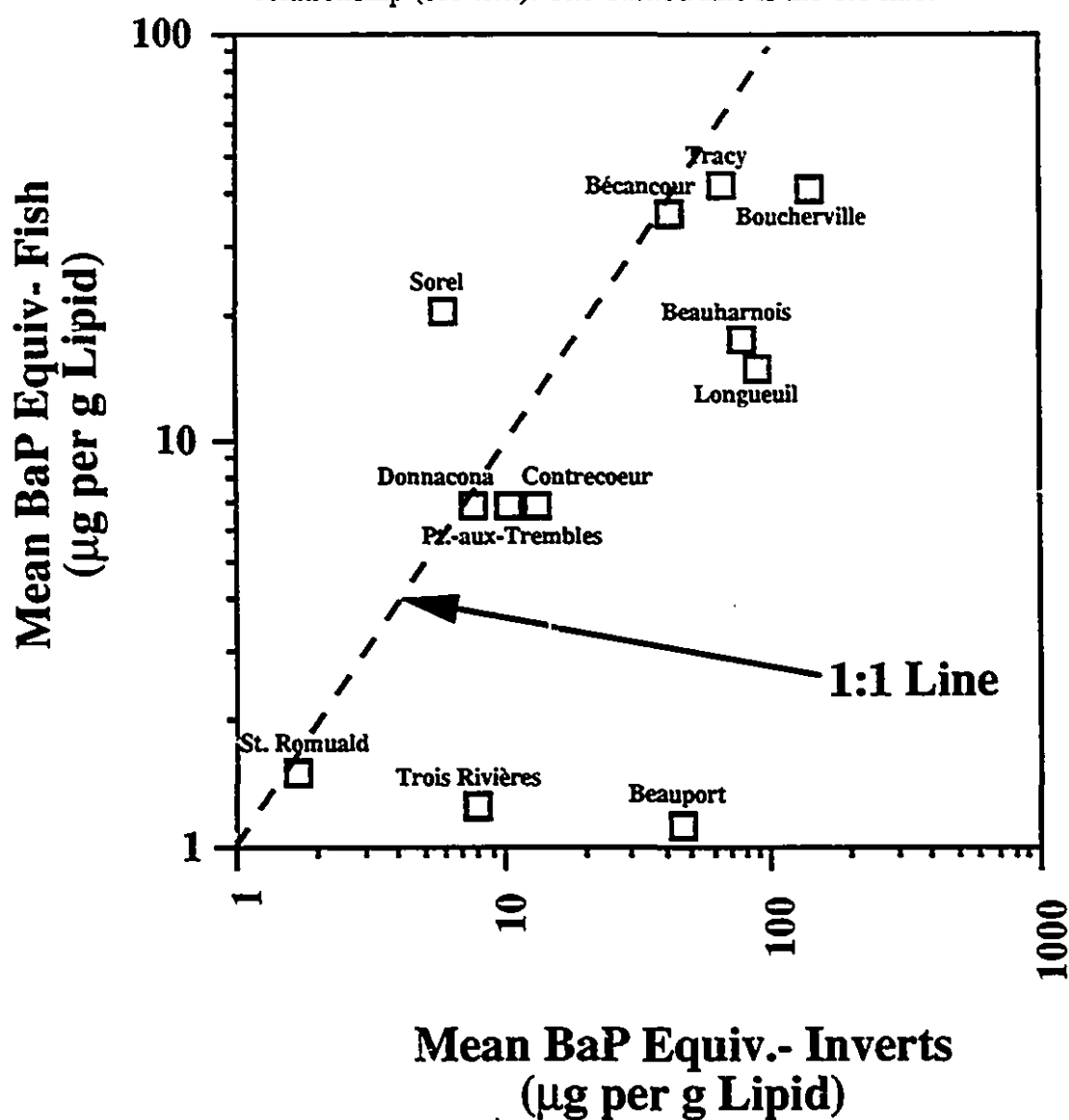
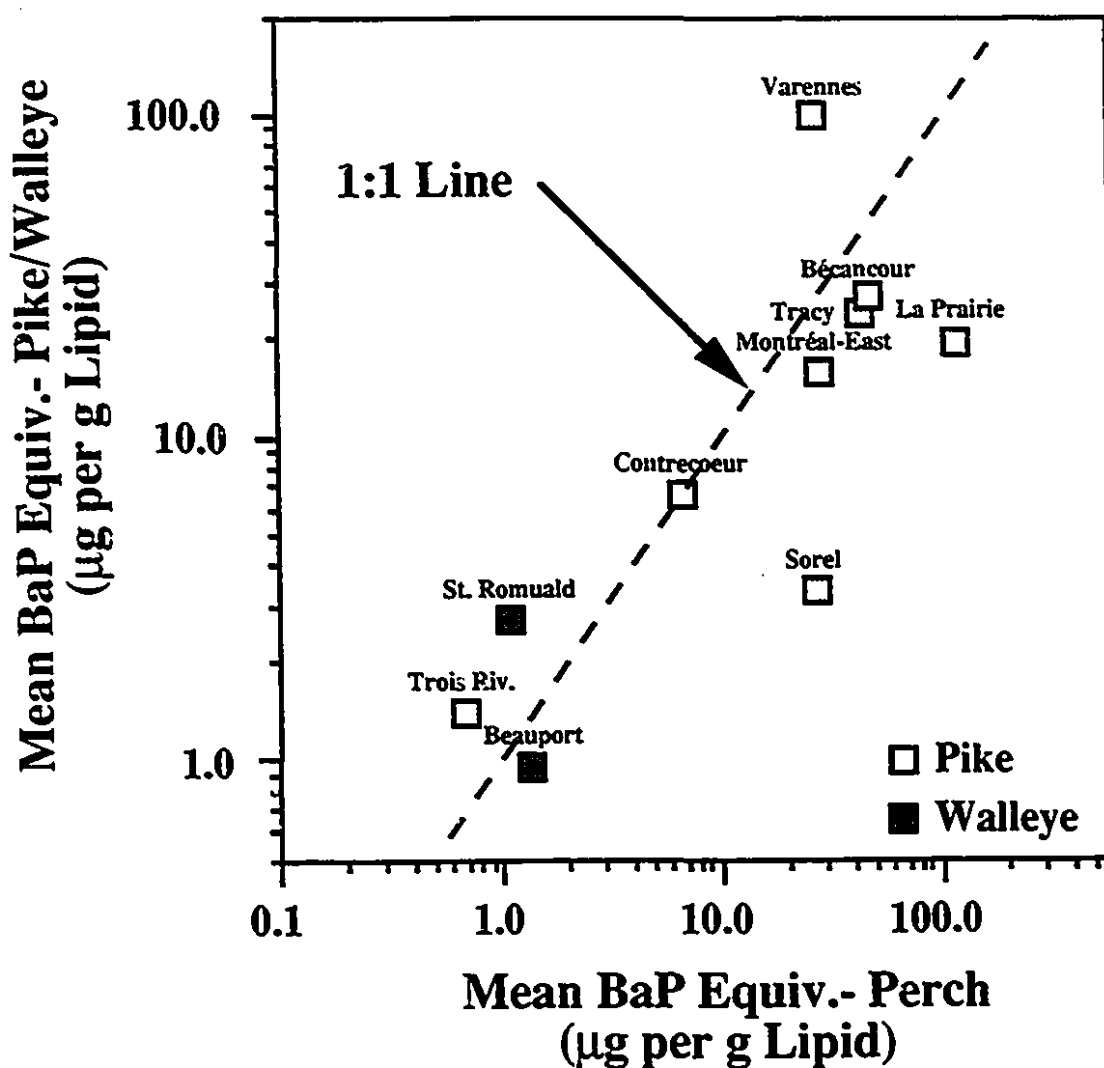


Figure 8. The relationship between the genotoxic contamination of piscivores such as pike and walleye and omnivores such as perch. Where several observations were available, values are arithmetic means. Numbers of observations ranged from 1 to 7. Linear regression results revealed a significant trend (see text). The dashed line is the 1:1 line.



Although the results reveal that lipid corrected mean perch values frequently exceed pike/walleye values, they do not indicate large differences in concentration. The results presented in Figure 5 indicated that the highest contamination levels are found in small perch. Lack of data prohibited the division of the perch data used to generate Figure 8 into the same categories as that used in Figure 5.

In our recent analyses of St. Lawrence and Saguenay river sediment samples we identified strong relationships between SOS genotoxicity and PAH contamination. To investigate similar relationships for biota extracts, PAH analyses were also conducted in this study. Table 4 provides a brief summary of the PAH results obtained.

Table 4. Maximum and mean concentrations of genotoxic PAHs detected in the tissue samples examined. All values are in ppb (wet wt.). Naphthalene, acenaphthylene, acenaphthene and fluorene are not included since there is no evidence that they are genotoxic. 3-Methylcholanthrene was never detected. Benzo(c)phenanthrene, benzo(ghi)perylene, dibenz(a,h)pyrene, dibenz(a,l)pyrene and dibenz(a,i)pyrene were not detected in fish tissues. 7,12-Dimethylbenz(a)anthracene was not detected in any invertebrate samples. Max. values are rounded to the nearest ppb. Mean values are rounded to the nearest 0.01 ppb. The last column summarizes the percentage of samples in which the substance was detected. When the compound was not detected, 0 ppb was used for the calculation of mean concentration.

Compound	Max. Concentration	Mean Concentration	% Detected
<i>Fish Tissues</i>			
Phenanthrene	10.9	1.00	46.0
Anthracene	3.0	0.15	7.8
Fluoranthene	2.0	0.07	5.3
Pyrene	2.0	0.04	2.6
Benz(a)anthracene	8.8	0.26	10.5
Chrysene	117.9	2.71	31.6
Benzo(bjk)fluoranthene [†]	3.9	0.09	2.6
7,12-Dimethylbenz(a)anthracene	2.0	0.05	3.9
Benzo(a)pyrene	5.9	0.08	1.3
Indeno(1,2,3-cd)pyrene	2.0	0.03	1.3

Table 4 continued.....

Dibenz(a,h)anthracene	1.9	0.03	1.3
<i>Invertebrate Tissues</i>			
Phenanthrene	15.6	4.92	95.2
Anthracene	4.8	0.61	19.0
Fluoranthene	60.4	8.94	90.5
Pyrene	39.6	6.95	76.2
Benzo(c)phenanthrene	15.8	0.75	4.8
Benz(a)anthracene	26.7	7.01	81.0
Chrysene	107.0	15.27	90.5
Benzo(bjk)fluoranthene [†]	38.6	6.00	57.1
Benzo(a)pyrene	16.4	1.90	38.1
Indeno(1,2,3-cd)pyrene	7.7	0.60	14.3
Dibenz(a,h)anthracene	1.9	0.09	4.8
Benzo(ghi)perylene	9.6	0.83	19.1
Dibenz(a,h)pyrene	0.9	0.05	4.8
Dibenz(a,l)pyrene	3.0	0.28	9.5
Dibenz(a,i)pyrene	1.9	0.92	4.8

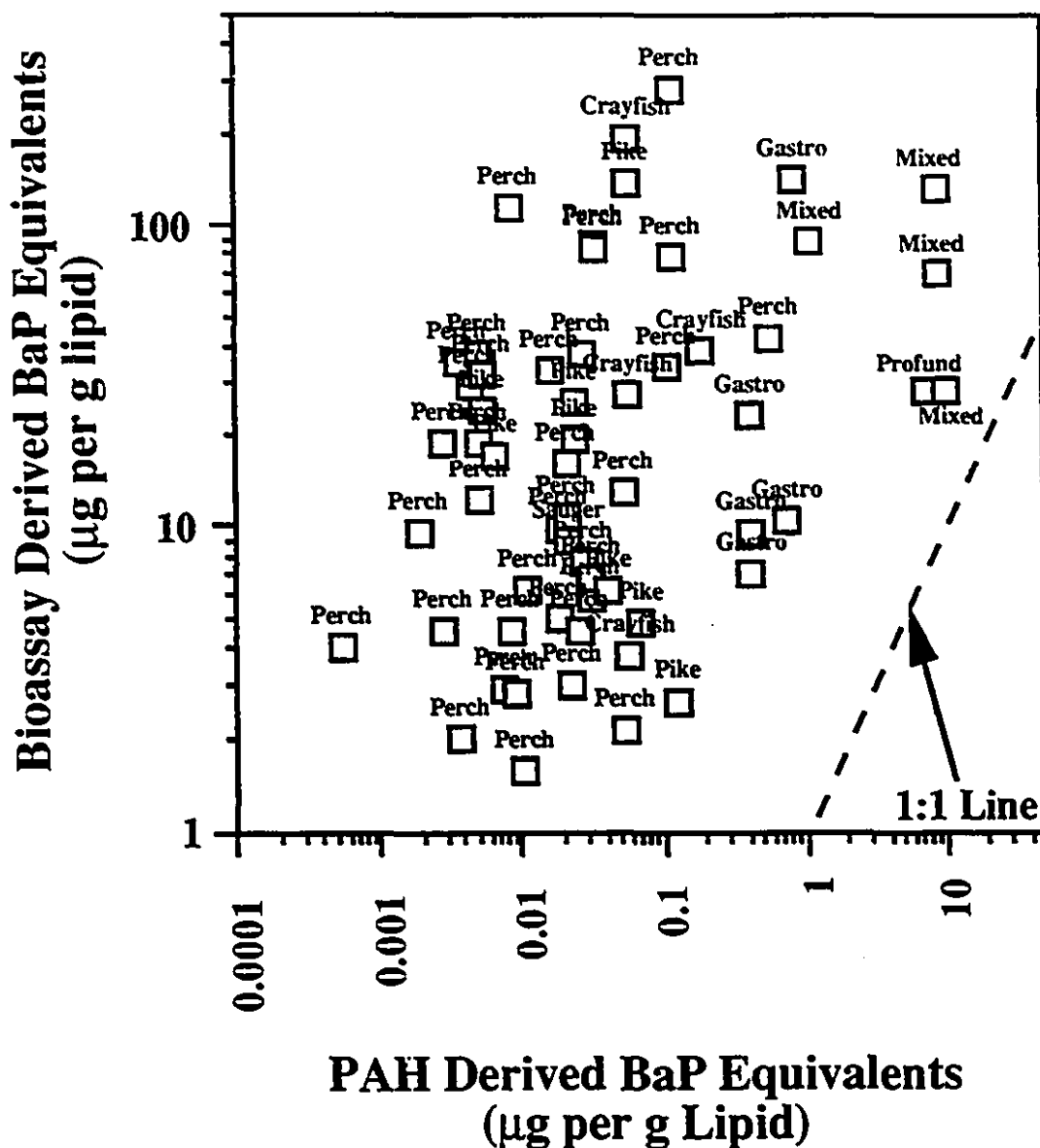
[†]Sum of benzo(b)fluoranthene, benzo(k)fluoranthene and benzo(j)fluoranthene.

The results in Table 4 indicate that while genotoxic PAHs were detected in fish tissues, concentrations are very low and frequently below detection. Invertebrate tissues also have detectable levels of genotoxic PAHs with mean concentrations that are several fold (3-175 fold) higher than that observed in fish.

To account for variations in the genotoxicity of PAHs examined, genotoxicity equivalency factors (GEF, Table 1) were used to convert PAH residues into their benzo(a)pyrene equivalent. The results obtained are illustrated in Figure 9. Linear regression analysis revealed a weak, but significant ($r^2 = 0.14$, F Ratio = 9.3, $p < 0.004$, $N = 61$) effect of PAH-derived concentration of BaP equivalents on the concentration of bioassay-derived BaP equivalents. However, the effect is not significant when each type of biota is considered separately. Concentrations of both bioassay derived and PAH-derived BaP equivalents are higher in invertebrates than fish. Therefore, it appears that the observed relationship is due to the same differences in biota contamination already

described. The results presented also indicate that the levels of bioassay derived BaP equivalents are on average 2-3 orders of magnitude greater than the corresponding PAH-derived value.

Figure 9. Tissue concentrations of bioassay-derived BaP equivalents vs. the concentration of PAH-derived BaP equivalents. Linear regression revealed a weak, but significant effect of PAH-derived concentration on bioassay-derived concentration (see text). The dashed line is the 1:1 line. Value labels used are identical to those in Figure 4.



DISCUSSION

Most published research efforts that employed bioassays to investigate genotoxins and mutagens in aquatic biota have been restricted to bivalve molluscs. They are sessile, long-lived suspension feeders known to accumulate genotoxic carcinogens such as BaP (Rodriguez-Ariza, 1992; Dunn, 1991). For example, Rodriguez-Ariza et al. (1992) detected oxidative mutagens in the tissues of three species of marine molluscs collected from Spanish coastal regions that are contaminated with a variety of toxic metals. Marvin et al. (1994) detected both frameshift and base-pair substitution mutagens in zebra mussels (*Dreissina polymorpha*) from Hamilton Harbour (Lake Ontario), an area renowned for PAH contamination (PAH concentration > 500 ppm dry wt., Marvin et al., 1993). To our knowledge only Kinae et al. (1981) investigated the accumulation of mutagens in fish. Using the Ames/*Salmonella* mutagenicity assay and a *Bacillus subtilis* DNA damage assay they detected both genotoxins and frameshift mutagens in the livers of spotted sea trout (*Nibea mitsukurii*) collected from areas that receive pulp and paper mill wastes.

The SOS Chromotest results obtained here reveal that aquatic biota in the St. Lawrence system do accumulate direct acting genotoxic substances. Although we did not thoroughly investigate the presence of genotoxins that require S9 activation, samples tested in the presence of S9 yielded a weaker, often negative response. Our analyses of soft-shell clams from the Saguenay Fjord revealed a similar reduction in potency upon addition of S9 (White et al., 1995b). Similar decreases in activity were observed by Rodriguez-Ariza et al. (1992) and Frezza et al. (1982). However, Marvin et al. (1994), Sparks et al. (1981) and Kira et al. (1989) observed increased mutagenicity in the presence of an S9 metabolic activation mixture. Both Marvin et al. (1994) and Kira et al. (1989) indicated that the sites examined are heavily contaminated with progenotoxic PAHs such as BaP.

The results presented in Figure 3 indicate that although both fish and invertebrate extracts contain SOS genotoxins, there is a difference in their relative ability to invoke the SOS response pathway. A lack of mechanistic understanding of SOS response induction make interpretation of these results difficult. However, they likely indicate a difference in the physical-chemical properties of the putative genotoxins accumulated in different types of biota. The results obtained indicate that molluscs, particularly *Mya arenaria* (White et al., 1995b), accumulate genotoxins that can elicit higher SOS Induction Factors than those accumulated by other biota. This difference is likely attributable to differences in the biochemistry of toxicant metabolism and excretion in aquatic biota. For example, metabolism of PAHs such as BaP in teleost fish is mediated by cytochrome P-450 monooxygenase. However, cytochrome P-450 monooxygenase is thought to play only a

minor role in molluscan PAH metabolism (Stegman and Lech, 1991). Analyses of pure compounds also reveal large differences in the level of SOS induction at which zero-order kinetics is manifested. For example, SOS Chromotest results for benzo(a)pyrene reveal saturation of the response pathway at induction factors between 3 and 8 (White, unpublished; Quillardet, unpublished). In contrast, compounds such as 4-nitroquinoline-1-oxide can produce SOS induction factors that easily exceed 25, with zero-order kinetics manifested at values above 60 (White, unpublished; Quillardet, unpublished).

Organisms with a high lipid content tended to be more contaminated with genotoxic organics. This result (see Figure 4) indicates that SOS genotoxins partition into the lipid pools of the exposed biota. Furthermore, the lipid trend is strong enough to justify lipid normalization. However, lipid content alone was only able to account for 22% of the variation in tissue concentrations of BaP equivalents. For a given lipid content the results revealed a wide range in observed tissue contamination. At 1% lipid for example, there is a 3 order of magnitude variation in genotoxic contamination. ANCOVA analyses confirmed that part of the variability can be accounted for by biota type and revealed that organisms at lower trophic levels have higher levels of contamination. When only the fish data were considered, ANCOVA revealed a similar pattern. In this case, fish occupying lower trophic levels were found to have higher tissue concentrations of genotoxins. While we do not claim to have detailed knowledge about the aquatic food chains of the St. Lawrence river, the results obtained here are supported by the trophic level assignments of Vander Zanden et al. (1995). The results presented in Figure 5 indicate that walleye have the lowest tissue levels of genotoxins, followed by pike and large perch, small perch and finally macroinvertebrates. Using diet studies and nitrogen stable isotope analyses Vander Zanden et al. (1995) revealed that walleye usually occupy a trophic position that is higher than other piscivores such as pike. Large perch and pike generally occupy a similar trophic position that is below walleye. Small perch generally occupy a position that is below pike and large perch, and slightly above crayfish.

The observed effect of trophic position on the tissue concentrations of genotoxins is quite different from that observed for persistent organic contaminants such as PCBs. Efficient transfer of PCBs from prey to predator results in a positive effect of trophic position on lipid normalized concentrations (Rasmussen et al., 1990). For genotoxic organics, the results obtained reveal a negative effect of trophic position on lipid normalized genotoxin concentrations. This *bio-diminution* likely results from the ability of fish to metabolize and excrete genotoxins contained in the tissues of their prey. Higher tissue burdens of genotoxins in invertebrates are likely the result of a lower trophic position and a decreased capacity for genotoxin metabolism and excretion. This decreased capacity for

genotoxin metabolism is likely related to difference in MFO (mixed function oxidase) activities. Fish MFO levels are usually much higher ($\approx 200 \text{ pmol min}^{-1} \text{ mg}^{-1}$) than those of invertebrates ($\approx 10\text{-}50 \text{ pmol min}^{-1} \text{ mg}^{-1}$) (Connor, 1984; Stegman and Lech, 1991; Dunn, 1991). Although it is not clear how increased MFO levels would effect SOS genotoxins, we would expect an increased ability for metabolism and excretion of organics to work against the biomagnification of genotoxins.

Although the observed effect of trophic position on genotoxin concentration is distinctly different from that observed for persistent substances, it is similar to that observed for more readily metabolized substances such as PAHs. For example, fish to sediment ratios for persistent, chlorinated hydrocarbons such as DDT and PCBs are in the 10^2 to 10^3 range (Connor, 1984). In contrast, PAHs have little tendency to biomagnify and generally exhibit biota to sediment ratios of 0.01 to 1.0. Eadie et al. (1982) found that oligochaetes from Lake Erie have PAH levels that are approximately 0.2 to 0.5 times sediment levels. Kauss and Hamdy (1991) found that tissue to sediment ratios for genotoxic PAHs in bivalve molluscs are generally less than 1. Varanasi et al. (1985) found that tissue to sediment ratios for PAHs in marine invertebrates ranged from 0.05 to 0.5, with higher values being associated with larger PAHs. Black et al. (1980) found that PAH levels in brown trout and white sucker from the Hershey river (Michigan) ranged from not detectable to about 1% of the sediment levels. Wide variations in the biota to sediment ratios for PAHs are generally attributed to a variable capacity for PAH metabolism and excretion (McElroy et al., 1989; Livingstone, 1994; James, 1989; Stegman, 1981). Comparisons of the tissue genotoxicity levels obtained here, with previously published suspended particulate matter and bottom sediment results (White et al., 1995a) are consistent with PAH-like degradability. Mean concentrations of SOS genotoxins in biota are about one-tenth (0.07 to 0.17)¹ the mean suspended particulate matter values (22 μg BaP equiv. per g dry wt. - no S9). Ratios to bottom sediment are slightly higher (0.25-1.25), but generally less than 1. Few researchers have published bioassay results that can be used to calculate similar biota to sediment ratios for genotoxic substances. The Ames/*Salmonella* results for zebra mussels and bottom sediment from Hamilton Harbour (Marvin et al., 1994; Marvin et al., 1993) provide invertebrate to bottom sediment ratios for mutagens that are less than 0.1. This low value can likely be attributed to the extremely high levels of PAHs in Hamilton Harbour sediments.

The surface water (SOS Chromotest) results of Langevin et al. (1992) indicate that the St. Lawrence river contains approximately 0.35 ppb BaP equivalents. The results presented

¹Ratios of biota (μg BaP per g dry tissue) to sediment (μg BaP per g dry sediment). Wet weight to dry weight conversion assumed 85% water content.

in Table 2 indicate that BCFs (bioconcentration factor) of genotoxins in the St. Lawrence system are $\approx 10^2 - 10^3$. Since Langevin et al. made an effort to sample in open waters not directly effected by local industrial and municipal discharges, this value should be considered a maximum. Several published regression models relate BCF values in fish and invertebrates to physical-chemical properties such as K_{ow} (Mackay, 1982; Neely et al., 1974; Veith et al., 1979; Bysshe, 1982). However, the ability of aquatic biota to metabolize and excrete genotoxic organics such as PAHs generally results in bioconcentration factors that are at least an order of magnitude lower than that of persistent organochlorines with similar K_{ow} 's (Pruell et al., 1986; Southworth et al. 1980; Southworth et al., 1981). We used a K_{ow} - BCF relationship for genotoxic PAHs² to calculate the K_{ow} of genotoxins accumulated by St. Lawrence river biota. The results indicate that the accumulated substances have K_{ow} 's in the 10^4 range. As a result we might expect a high proportion of surface water genotoxins to be present in the dissolved, rather than particle-bound state (e.g. at 5 mg per L suspended solids $\approx 95\%$ dissolved). The low tendency for particle sorption may result in increased bioavailability of genotoxins (Allan, 1986). It is interesting to note that the calculated K_{ow} is similar to that inferred from the sorption partition coefficient of genotoxins in domestic wastewaters ($K_d = 8000$, $K_{ow} = 2 \times 10^4$, White et al., 1995e). In addition, the mass balance calculations of White et al. (1995f) indicated that the majority of direct acting SOS genotoxins in the surface waters of the St. Lawrence system are likely domestic in origin. While this correspondence is interesting, it should be noted that most of the local loadings at the sites examined may not be domestic in origin. Thus it is not possible to state whether the substances accumulated by biota are industrial or domestic in origin.

The results presented in Figures 4 and 5 and equations (1) and (2) do not address the fact that the biota collected inhabit regions with varying levels of contamination. Some of the unexplained variation in tissue contamination must be attributable to these differences in site contamination. The results presented in Figure 6 indicates that while there is little evidence of genotoxicity at clean sites, sites that receive industrial and municipal wastes show a negative effect of loading on biota contamination. Lower mutagenicity of tissue extracts at more contaminated sites was also observed by Rodriguez-Ariza et al. (1992). They attributed the result to increased levels of detoxifying enzymes such as glutathione-S-transferase and glutathione peroxidase in organisms that inhabit more contaminated areas. Further research by Rodriguez-Ariza et al. (1995) and others (e.g. Bagnasco et al., 1991; Kurelec et al., 1979) revealed that fish inhabiting contaminated areas also have an enhanced

²Linear regression function of Log K_{ow} vs. Log BCF was calculated using data of Mackay (1982) and de Voogt et al. (1991).

ability to metabolize and activate promutagenic substances. This enhanced ability has been attributed to increased MFO activity in fish and other organisms at polluted sites (Stegman and Lech, 1991). For example, Britvic et al. (1993) observed that carp (*Cyprinus carpio*) inhabiting polluted environments had increased bile fluorescence, increased liver BaP monooxygenase activity and provided liver S9 preparations that had an enhanced ability to activate BaP *in vitro*. A similar enhancement of the ability to metabolize genotoxic substances may be occurring at the sites investigated here. Biota inhabiting sites that receive high genotoxic loadings (e.g. Trois Rivières and Contrecoeur) may have increased phase I and/or phase II enzyme activities, and a concomitant increased ability to metabolize and excrete genotoxic substances. However, since metabolism also results in genotoxin activation, we might expect genotoxic effects (DNA adducts, mutations, strand breaks, etc) to be higher in organisms from sites where genotoxic loadings are very high.

Although the results failed to demonstrate a significant relationship between regional genotoxic loading and biota contamination, the results presented in Figure 7 and 8 and equation (3) and (4) indicate that within individual sites the results are consistent. These results permit comparisons of the biota examined while simultaneously controlling for variations in site contamination. In Figure 7 and equation (3) we confirmed that mean fish contamination is generally about half an order of magnitude lower than corresponding invertebrate levels. The results presented in Figure 8 and equation (4) revealed little difference between the mean perch and mean pike/walleye contamination. The discrepancy between this result and that presented in Figure 4 and equation (2) is likely due to the fact that most of the perch results used to calculate mean contamination levels at each site correspond to specimens greater than 15 cm in length ($\approx 70\%$).

PAH analyses summarized in Table 4 indicate that fish tissues are far more contaminated than invertebrate tissues. Ratios of invertebrate PAH concentration to fish PAH concentration (>10) are similar to those obtained by Black et al. (1984). Many researchers have identified similar patterns that are generally attributed to the aforementioned differential abilities for PAH metabolism and excretion. The PAH results reveal a similar pattern of bio-diminution to that seen for genotoxins. Although the results presented in Figure 9 revealed a weak effect of PAH-derived BaP equivalents on bioassay-derived BaP equivalents, the relationship is dominated by the differences between invertebrates and fish that were summarized in Figure 5. However, since bioassay derived concentration values were calculated from direct acting genotoxicity measurements, and the PAHs investigated all require S9 activation, the results obtained cannot imply that PAHs are responsible for the observed differences between fish and invertebrates. The fact that PAH values follow a bio-diminution trend that is similar to that observed for genotoxins

suggests that some putative genotoxins have physical-chemical properties that are similar to PAHs. In addition to the lack of an effect of PAH concentration on extract genotoxicity, the levels of PAH-derived concentrations are unable to account for more than a small fraction of the observed SOS genotoxicity.

Despite an absence of supporting data, Moutschen (1985) predicts the "amplified effects" that would result from genotoxin bioaccumulation and biomagnification. The goal of this study was to investigate the tendency for genotoxins in the St. Lawrence system to bioaccumulate and biomagnify. The results obtained confirmed that genotoxic organic substances do accumulate in the tissues of aquatic biota. Tissue levels are 100 to 1000 times higher than ambient water concentrations. However, despite this accumulation, the results indicate that genotoxins are much more readily degraded than persistent chlorinated organics such as PCBs and DDT. Comparisons with PAHs indicate that they have similar degradability. Comparisons of different types of biota indicate that genotoxins are bio-diminished rather than bio-magnified, with highest levels in invertebrates that occupy a low trophic position and have a reduced capacity for metabolism and excretion of organics. Invertebrate contamination may constitute a serious health hazard to human consumers. Several researchers (e.g. Dunn, 1991) have voiced concern about the carcinogenic hazard of consuming invertebrates that have a tendency to accumulate genotoxic organics.

Moutschen (1985) also expressed particular concern for substances that can exert genotoxic effects at far lower doses than those required for other toxic endpoints that are more readily observable. Although we did not investigate genotoxic effects, previous research efforts have demonstrated that these effects can be severe. Many researchers have documented the high frequency of neoplasia in fish and invertebrates that inhabit areas contaminated with genotoxic and carcinogenic PAHs (Mix, 1986; Malins et al., 1984; Landahl et al., 1990; Gardner et al., 1987; Metcalfe et al., 1990). However, adverse effects are certainly not limited to neoplastic transformation. Kurelec (1993) proposes a wide range of other genotoxic effects including growth inhibition, premature aging, tissue degeneration and immunosuppression. The results obtained here suggest that genotoxic effects are likely to be most pronounced in small perch. These fish likely possess the ability to activate genotoxins assimilated from contaminated invertebrates. Examinations of genotoxic effects (e.g. neoplasia, DNA damage, chromosomal aberrations - see Dixon, 1982; di Giulio et al., 1993; Nacci and Jackim, 1989) and biomarkers of genotoxin metabolism (e.g. genotoxin metabolites in bile, DNA adducts - see Krahn et al. 1986; Britvic et al., 1993; Varanasi et al., 1989) in small perch and invertebrates from the St. Lawrence system seems a promising area for further research.

ACKNOWLEDGEMENTS

We are grateful to several people for their support and assistance. Pierre Fournier of the Quebec Ministry of Environment and Wildlife (MEF) furnished the permits required to collect fish for scientific research. Regional MEF representatives provided additional assistance and advise. Jay Leopkey, Brigid Payne, Cornelia den Heyer and Brian Walker provided excellent technical assistance in the field and the laboratory. Chain pickerel from L. Memphremagog were graciously donated by Jake Vander Zanden. Northern Pike from Lake Onatchiway were donated by Christian Blaise. The study was supported by a St. Lawrence Center-Natural Sciences and Engineering Research Council of Canada research partnership grant to JBR and a Natural Sciences and Engineering Research Council of Canada doctoral research fellowship to PAW.

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GENERAL CONCLUSION

This thesis has revealed a great deal of information about the sources and fates of genotoxic organic contaminants in the St. Lawrence river. Although genotoxins entering the river appear to come from a wide range of sources, domestic wastewaters have particularly high daily loadings. As a result they account for a large portion of the surface water genotoxicity observed by Langevin et al. (1992). However, it should be noted that these results refer to open waters that are not heavily impacted by local industrial sources. Locally, industrial wastewater sources appear to have an impact on the receiving system, with the genotoxicity of collected samples being empirically related to local loading or potency. Suspended sediments in these areas are direct-acting and appear to be a diluted version of the particulate material present in the effluents themselves. Bottom sediment genotoxicity is related to local loading, with accumulated substances requiring S9 metabolic activation. The results suggest that direct-acting substances are more readily degraded and may be converted into more stable pro-genotoxic compounds that accumulate in sedimented material.

Biota inhabiting industrialized regions are accumulating direct acting genotoxins, with more contaminated sites yielding less contaminated specimens. Comparisons of biota from different trophic levels indicates that genotoxins detected with the SOS Chromotest have an ecological behaviour similar to that of readily metabolized organic contaminants such as PAHs. They are not efficiently transferred from prey to predator and increases in trophic position are associated with decreased levels of tissue genotoxicity. Although the results presented indicate that the accumulated substances have a K_{ow} in the 10^4 range, this value does not necessarily reflect the physical-chemical properties of the substances to which the organisms were exposed.

In each chapter of this thesis comparative analyses of a wide range of samples, collected from a wide range of sites, permitted general statements about the fate of industrial genotoxins in aquatic systems. Although other researchers have investigated the genotoxicity of surface waters, sediments, biota and industrial wastes, the research projects typically ask questions about individual compounds or particularly contaminated samples. These questions are then addressed using detailed, mechanistic investigations. Peters (1986) states that while such investigations can be productive, they are often "....discursive, explanatory idealizations" that "transpose badly to other systems". While comparative analyses permitted general statements about the behaviour of genotoxic substances, this generality was achieved by sacrificing descriptive precision.

The results presented in this thesis have revealed that bioassays can be used to

investigate the sources and fates of toxic contaminants without any *a priori* information about identity or physical-chemical properties. In this work, the endpoint employed was the error prone DNA repair pathway of *Escherichia coli*. However, the research strategy should prove equally efficacious for other toxic endpoints. In all cases, the results obtained must be interpreted in the context of the endpoint employed for toxicity detection. In this respect, empirical analyses of published data were used to explore the connotation of a positive result on the SOS Chromotest.

Although this research has answered many questions, it has also created a large number of new ones. Many questions concern the chemical identity of genotoxins present in particularly interesting samples. Bioassay-directed fractionation techniques (Schuetzle and Lewtas, 1986; Alfheim et al., 1984) can be used to isolate and ultimately identify the putative genotoxins in these samples. Among the more interesting samples are effluents from municipal wastewater treatment facilities. Bioassay-directed chemical analyses and more detailed mass balance investigations will indicate the origins, fates and identity of genotoxins in domestic wastewaters.

It should also prove interesting to conduct a more in depth analysis of genotoxins accumulated by aquatic biota, particularly macroinvertebrates. The results obtained indicate that mixed invertebrate samples containing insect larvae and oligochaetes yielded particularly genotoxic extracts. Although several researchers have investigated the ability of molluscs and crustaceans to metabolize and excrete genotoxic substances (James, 1989; Livingstone et al., 1989), few have investigated aquatic insects and oligochaetes (Neff, 1979). Although some studies have detected high levels of microsomal mixed function oxidases in insects (e.g. Kreiger and Wilkinson, 1969), most taxa have not been examined.

Finally, further research must examine genotoxic effects on exposed biota, particularly those that inhabit regions which receive high genotoxic loadings. Conventional approaches would call for investigations of effects on individuals, particularly those mediated by somatic cell mutation. This focus on effects related to damage of somatic cell DNA has resulted in genotoxicity and mutagenicity becoming more or less synonymous with somatic cell mutation and cancer (Zeiger, 1995). However, in an ecological context, genotoxic effects on somatic cells may be of little consequence, particularly if they become clinically visible long after sexual maturity. For example, typical latency periods for the formation of malignant tumours results in losses from a population that are most dramatic for the older age classes (e.g. see *Ictalurus nebulosus* tumor frequency data of Maccubbin and Erasing, 1991). Genotoxic effects on germ cells are likely to be more consequential, with alterations of the germ cell mutation rate effecting the dynamics of entire populations. Recent publications have begun to investigate the consequences of population level effects related

to mutagenic pollutants. The computer simulations of Lynch et al. (1995) indicate that a doubling of the deleterious mutation rate will be expected to reduce the longevity of a population by about 50%, with small populations being particularly vulnerable to extinction via "mutational meltdown". Moreover, deleterious mutations introduced into a population will remain in the gene pool long after the source of genotoxic pollutants is extinguished. Further research in this area will prove difficult and challenging, but equally interesting and profitable.

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APPENDIX 1

Program to control Biomek™ 1000 Automated Laboratory Workstation

Introductory Notes:

The method was written using the Genesis™ software version 2.11. To streamline the assay microplates were processed in batches of four. The program, divided into three subroutines, processes one batch of four plates. The first subroutine distributes media and *E. coli* PQ37 culture. The second subroutine carries out the required 2-fold serial dilutions. The third subroutine resuspends exposed cells in buffer and chromogenic reagent.

Subroutine #1: EFFMED4

Function: Config Change

Tips: P250 Tips (New)
 Tray 1A: Single well half module
 Tray 1C: Single well half module
 Tray 2: 1 well
 Tray 3: 96 well flat bottom
 Tool A: MP20
 Tool B: MP200
 Tool C: Empty
 Tool D: P200
 Parameter set: [Unspecified]

Function: Loop

Loop 4 times
 Log: Clock-Elapsed

Function: Config Change

Tips: P250 Tips
 Tray 1A: Single well half module
 Tray 1C: Single well half module
 Tray 2: 1 well
 Tray 3: 96 well flat bottom
 Tool A: MP20
 Tool B: MP200
 Tool C: Empty
 Tool D: P200
 Parameter set: [unspecified]

Function: Message

Get Next Plate Ready for Media - Keep Track of Pipette Tips

Function: Pause-Continue

Function: Pipette

Volume: 5 microliters Tool: MP20
 Src: Tray 2 By Col A1-A1 Repeat
 Dest: Tray 3 Box, Col A2-H6 Stop
 Source Hgt: Bottom Dest. Hgt: Bottom
 Rate: 3 To Deliver Tip Touch at Dest
 No Tip Change No Prewet
 Log: Clock-Elapsed

Function: Pipette

Volume: 5 microliters Tool: MP20
 Src: Tray 2 By Col A1-A1 Repeat

Dest: Tray 3 Box, Col A8-H12 Stop
 Source Hgt: Bottom Dest. Hgt: Bottom
 Rate: 3 To Deliver Tip Touch at Dest
 No Tip Change No Prewet
 Log: Clock-Elapsed

Function: Pause-Continue

Function: Pipette

Volume: 190 microliters Tool: P200
 Src: Tray 1A By Row A1-A1 Stop
 Dest: Tray 3 By Row H1-H1 Stop
 Source Hgt: Bottom Dest. Hgt: Top
 Rate: 3 To Contain: Blowout Tip Touch at Dest
 No Tip Change No Prewet
 Log: Clock-Elapsed

Function: Pipette

Volume: 95 microliters Tool: P200
 Src: Tray 1A By Row A1-A1 Stop
 Dest: Tray 3 By Row H2-H6 Reps 5 Stop
 Source Hgt: Bottom Dest. Hgt: Top
 Rate: 3 To Contain: Blowout Tip Touch at Dest
 No Tip Change No Prewet
 Log: Clock-Elapsed

Function: Tip Change

Function: Pipette

Volume: 190 microliters Tool: P200
 Src: Tray 1A By Row A2-A2 Repeat
 Dest: Tray 3 By Col Pattern #1 Stop
 Source Hgt: Bottom Dest. Hgt: 25%
 Rate: 3 To Contain: Blowout Tip Touch at Dest
 No Tip Change No Prewet
 Log: Clock-Elapsed

Function: Pipette

Volume: 95 microliters Tool: MP200
 Src: Tray 1A By Row A2-A2 Repeat
 Dest: Tray 3 Box, Col A2-H6 Stop
 Source Hgt: Bottom Dest. Hgt: 25%
 Rate: 3 To Contain: Blowout Tip Touch at Dest
 No Tip Change No Prewet
 Log: Clock-Elapsed

Function: Tip Change

Function: Pipette

Volume: 95 microliters Tool: MP200
 Src: Tray 1A By Row A2-A2 Repeat
 Dest: Tray 3 Box, Col A8-H12 Stop
 Source Hgt: Bottom Dest. Hgt: 25%
 Rate: 3 To Contain: Blowout Tip Touch at Dest
 No Tip Change No Prewet
 Log: Clock-Elapsed

Function: Next

Subroutine #2: SERIAL4**Function: Config Change**

Tips: P250 Tips (New)
 Tray 1: 96 well flat bottom
 Tray 2: Empty
 Tray 3: 1 Well
 Tool A: Empty
 Tool B: MP200
 Tool C: Empty
 Tool D: P200
 Parameter set: [Unspecified]

Function: Loop

Loop 4 times
 Log: Clock-Elapsed

Function: Config Change

Tips: P250 Tips
 Tray 1: 96 well flat bottom
 Tray 2: Empty
 Tray 3: 1 Well
 Tool A: Empty
 Tool B: MP200
 Tool C: Empty
 Tool D: P200
 Parameter set: [Unspecified]

Function: Message

Get Ready For Serial Dilutions

Function: Pause-Continue**Function: Pipette**

Volume: 100 microliters Tool: MP200
 Loc: Tray 1 Box, Col A1-H6
 Serial Transfer
 Source Hgt: Bottom Dest. Hgt: Bottom
 Rate: 2 To Contain: Blowout Tip Touch at Src
 No Tip Change No Prewet
 Mixing Low at Src 75 μ L 5 times
 Log: Clock-Elapsed

Function: Pipette

Volume: 100 microliters Tool: MP200
 Src: Tray 1 Box, Col A6-H6 Stop
 Dest: Tray 3 Box, Col A1-A1 Stop
 Source Hgt: Bottom Dest. Hgt: Bottom
 Rate: 2 To Contain: Blowout Tip Touch at Src
 No Tip Change No Prewet
 Mixing Low at Src 75 μ L 5 times
 Log: Clock-Elapsed

Function: Pipette

Volume: 100 microliters Tool: MP200
 Loc: Tray 1 Box, Col A7-H12
 Serial Transfer

Source Hgt: Bottom Dest. Hgt: Bottom
 Rate: 2 To Contain: Blowout Tip Touch at Src
 No Tip Change No Prewet
 Mixing Low at Src 75 μ L 5 times
 Log: Clock-Elapsed

Function: Pipette

Volume: 100 microliters Tool: MP200
 Src: Tray 1 Box, Col A12-H12 Stop
 Dest: Tray 3 Box, Col A1-A1 Stop
 Source Hgt: Bottom Dest. Hgt: Bottom
 Rate: 2 To Contain: Blowout Tip Touch at Src
 No Tip Change No Prewet
 Mixing Low at Src 75 μ L 5 times
 Log: Clock-Elapsed

Function: Tip Change

Function: Next

Subroutine #3: RESUSP4

Function: Config Change

Tips: P250 Tips (New)
 Tray 1: 1 Well
 Tray 2: 1 Well
 Tray 3: 96 well flat bottom
 Tool A: Empty
 Tool B: MP200
 Tool C: Empty
 Tool D: P200
 Parameter set: [Unspecified]

Function: Loop

Loop 4 times
 Log: Clock-Elapsed

Function: Config Change

Tips: P250 Tips
 Tray 1: 1 Well
 Tray 2: 1 Well
 Tray 3: 96 well flat bottom
 Tool A: Empty
 Tool B: MP200
 Tool C: Empty
 Tool D: P200
 Parameter set: [Unspecified]

Function: Message

Get Ready To Resuspend in Chromogen

Function: Pause-Continue

Function: Pipette

Volume: 100 microliters Tool: MP200
 Src: Tray 2 By Col, A1-A1 Stop
 Dest: Tray 3 Box, Col A1-H12 Reps 12 Stop
 Source Hgt: Bottom Dest. Hgt: 75%

Rate: 3 To Contain: Blowout No Tip Touch
No Tip Change No Prewet
Log: Clock-Elapsed

Function: Pipette

Volume: 100 microliters Tool: MP200
Src: Tray 2 By Col, A1-A1 Stop
Dest: Tray 3 Box, Col A1-H12 Reps 12 Stop
Source Hgt: Bottom Dest. Hgt: 75%
Rate: 2 To Contain: Blowout No Tip Touch
No Tip Change No Prewet
Log: Clock-Elapsed

Function: Tip change

Function: Next

Method Ends

APPENDIX 2

Program to control Titertek Multiskan MCC/340 microplate spectrophotometer--

(Italicized text are comments)

100 Remark Dual 405 - 620 *Select filters for 405 and 620 nm readings*
200 Filter 2 *405 nm Filter*
300 Air blank *Blank instrument on air*
400 Read plate Temp1.plf *Read plate @ 405 nm and save as temporary file*
500 Let Z1=Mean(H1:H6) *Calculate mean OD value for reagent blank wells*
600 Let A1:H12=(A1:H12)-Z1 *Subtract mean reagent blank value from all readings*
700 Save Data P405_.plf *Save data file as P405_XXX.plf, where XXX is plate number*
800 Wait 0.0 secs *No delay before 620 nm readings*
900 Filter 7 *620 nm Filter*
1000 Air Blank
1100 Read Plate Temp2.plf *Read plate @ 620 nm and save as temporary file*
1200 Let Z2=Mean(H1:H6) *Calculate mean OD value for reagent blank wells*
1300 Let A1:H12=(A1:H12)-Z2 *Subtract mean reagent blank value from all readings*
1400 Save data P620_.plf *Save data file as P405_XXX.plf, where XXX is plate number*
1500 Wait 0.0 secs
1600 Go to Line 100

APPENDIX 3

Data used to generate the Figures included in Chapter 1.

1) Time series data for Figure 2. Diesel particulate extract was tested at 3.125 mg equivalent starting material per assay mL, no S9 activation. Urban air particulates were tested at 25 mg equivalent starting material per assay mL.

Sample	Incubation Time (minutes)	Optical Density @ 620 nm
Diesel Particulates	0	0.049
Diesel Particulates	5	0.051
Diesel Particulates	10	0.047
Diesel Particulates	15	0.045
Diesel Particulates	20	0.042
Diesel Particulates	27	0.042
Diesel Particulates	33	0.042
Diesel Particulates	38	0.040
Diesel Particulates	43	0.043
Diesel Particulates	48	0.044
Diesel Particulates	53	0.046
Diesel Particulates	58	0.046
Diesel Particulates	63	0.049
Diesel Particulates	70	0.049
Diesel Particulates	120	0.077
Diesel Particulates	180	0.099
Air Particulates	0	0.221
Air Particulates	5	0.214
Air Particulates	10	0.213
Air Particulates	15	0.207
Air Particulates	20	0.208
Air Particulates	27	0.208
Air Particulates	33	0.205
Air Particulates	38	0.206
Air Particulates	43	0.204
Air Particulates	48	0.207
Air Particulates	53	0.205
Air Particulates	58	0.204
Air Particulates	63	0.207
Air Particulates	70	0.207
Air Particulates	120	0.216
Air Particulates	180	0.224

2) Data used for Figure 3. Concentration-response data documenting the effect of cell density on the genotoxicity of 4-NQO and Diesel Particulate extract.

Sample	Concentration (μ g per assay mL)	Cell density (cfu per mL)	Mean Induction Factor	Standard Error
4-NQO	0.0391	5.00E+06	2.831	0.061
4-NQO	0.0781	5.00E+06	5.883	0.156
4-NQO	0.1563	5.00E+06	12.530	0.366

4-NQO	0.3125	5.00E+06	38.217	2.545
4-NQO	0.6250	5.00E+06	100.721	4.765
4-NQO	1.2500	5.00E+06	27.724	1.176
4-NQO	0.0390	1.00E+07	1.698	0.067
4-NQO	0.0780	1.00E+07	3.099	0.104
4-NQO	0.1560	1.00E+07	8.176	0.283
4-NQO	0.3120	1.00E+07	22.560	1.539
4-NQO	0.6250	1.00E+07	42.901	5.643
4-NQO	1.2500	1.00E+07	51.917	1.580
4-NQO	0.0390	2.00E+07	1.753	0.172
4-NQO	0.0780	2.00E+07	2.657	0.127
4-NQO	0.1560	2.00E+07	5.861	0.107
4-NQO	0.3120	2.00E+07	12.483	0.348
4-NQO	0.6250	2.00E+07	20.404	1.508
4-NQO	1.2500	2.00E+07	17.130	3.854

(mg per assay
mL)

Diesel	0.1953	5.00E+06	11.908	0.172
Diesel	0.3906	5.00E+06	21.441	0.127
Diesel	0.7813	5.00E+06	21.373	0.107
Diesel	1.5625	5.00E+06	18.864	0.348
Diesel	3.1250	5.00E+06	15.054	1.508
Diesel	6.2500	5.00E+06	3.733	3.854
Diesel	0.1950	1.00E+07	8.601	0.328
Diesel	0.3910	1.00E+07	20.221	1.990
Diesel	0.7810	1.00E+07	41.360	5.113
Diesel	1.5620	1.00E+07	37.265	1.670
Diesel	3.1250	1.00E+07	17.484	0.720
Diesel	6.2500	1.00E+07	6.174	0.307
Diesel	0.1950	2.00E+07	5.501	0.164
Diesel	0.3910	2.00E+07	8.793	0.215
Diesel	0.7810	2.00E+07	14.639	0.582
Diesel	1.5620	2.00E+07	18.272	1.446
Diesel	3.1250	2.00E+07	13.250	1.073
Diesel	6.2500	2.00E+07	5.210	0.874

3) Data used to generate Figure 4. Concentration-response data documenting the effect of chromogen incubation time density on the response of 4-NQO, MNNG, Diesel Particulate extract and PAH Mixture from Coal Tar.

Sample	Concentration (μ g per assay mL)	Incubation Time (min)	Mean SOS Induction Factor	Standard Error
Mitomycin C	0.0313	30	3.364	0.348
Mitomycin C	0.0625	30	5.174	0.123
Mitomycin C	0.1250	30	8.517	0.183
Mitomycin C	0.2500	30	12.998	0.300
Mitomycin C	0.5000	30	16.219	0.256
Mitomycin C	1.0000	30	21.102	0.126
Mitomycin C	0.0310	60	3.979	0.007
Mitomycin C	0.0620	60	5.954	0.403
Mitomycin C	0.1250	60	9.462	0.642
Mitomycin C	0.2500	60	12.486	0.728
Mitomycin C	0.5000	60	14.594	0.554
Mitomycin C	1.0000	60	18.762	0.441

Mitomycin C	0.0310	90	4.062	0.233
Mitomycin C	0.0620	90	5.811	0.345
Mitomycin C	0.1250	90	8.081	0.723
Mitomycin C	0.2500	90	9.899	1.547
Mitomycin C	0.5000	90	10.366	1.369
Mitomycin C	1.0000	90	12.712	0.573
MNNG	0.6250	30	1.223	0.063
MNNG	1.2500	30	1.543	0.034
MNNG	2.5000	30	1.947	0.015
MNNG	5.0000	30	2.516	0.088
MNNG	10.0000	30	3.527	0.213
MNNG	20.0000	30	4.293	0.124
MNNG	0.6250	60	1.238	0.155
MNNG	1.2500	60	1.607	0.085
MNNG	2.5000	60	1.834	0.063
MNNG	5.0000	60	2.393	0.153
MNNG	10.0000	60	3.418	0.067
MNNG	20.0000	60	4.687	0.306
MNNG	0.6250	90	1.126	0.021
MNNG	1.2500	90	1.459	0.011
MNNG	2.5000	90	2.018	0.114
MNNG	5.0000	90	2.411	0.148
MNNG	10.0000	90	3.643	0.196
MNNG	20.0000	90	4.754	0.238

(mg per assay
mL)

Diesel Particulates	0.1950	60	10.679	0.878
Diesel Particulates	0.3910	60	21.567	1.948
Diesel Particulates	0.7810	60	28.752	1.465
Diesel Particulates	1.5620	60	79.029	8.455
Diesel Particulates	3.1250	60	45.891	5.635
Diesel Particulates	6.2500	60	9.255	0.330
Diesel Particulates	0.1950	90	8.267	0.352
Diesel Particulates	0.3910	90	9.069	0.386
Diesel Particulates	0.7810	90	10.705	1.358
Diesel Particulates	1.5620	90	8.038	2.232
Diesel Particulates	3.1250	90	18.763	2.447
Diesel Particulates	6.2500	90	7.760	0.316
Diesel Particulates	0.1950	120	6.237	0.160
Diesel Particulates	0.3910	120	7.570	0.992
Diesel Particulates	0.7810	120	7.655	0.759
Diesel Particulates	1.5620	120	7.126	1.921
Diesel Particulates	3.1250	120	10.168	1.125
Diesel Particulates	6.2500	120	6.805	0.202
PAH From Tar	0.7810	60	1.201	0.055
PAH From Tar	1.5620	60	1.804	0.121
PAH From Tar	3.1250	60	1.962	0.051
PAH From Tar	6.2500	60	2.016	0.039
PAH From Tar	12.5000	60	1.870	0.069
PAH From Tar	25.0000	60	1.570	0.042
PAH From Tar	0.7810	90	1.253	0.047
PAH From Tar	1.5620	90	1.819	0.103
PAH From Tar	3.1250	90	1.921	0.044
PAH From Tar	6.2500	90	2.014	0.033

PAH From Tar	12.5000	90	1.908	0.066
PAH From Tar	25.0000	90	1.528	0.003
PAH From Tar	0.7810	120	1.238	0.052
PAH From Tar	1.5620	120	1.770	0.086
PAH From Tar	3.1250	120	1.969	0.053
PAH From Tar	6.2500	120	1.959	0.040
PAH From Tar	12.5000	120	1.855	0.066
PAH From Tar	25.0000	120	1.488	0.006

4) Data used to generate Figure 5.

Sample/ Activation	Time (a:mins, b:hrs)	Mean SRIP (IF per mg orig. sample)	Std. Error
Figure 5a - Chromo Time Experiment			
Diesel No S9	60	24.600	12.420
PAH Mix No S9	60	21.420	8.770
Diesel +S9	60	4.330	0.857
PAH Mix+S9	60	19.130	5.440
Air Part. + S9	60	0.158	0.030
Diesel No S9	90	5.770	2.500
PAH Mix No S9	90	18.875	7.580
Diesel +S9	90	2.830	0.381
PAH Mix+S9	90	16.120	4.970
Air Part. + S9	90	0.122	0.024
Diesel No S9	120	3.730	1.300
PAH Mix No S9	120	16.920	9.998
Diesel +S9	120	2.450	0.361
PAH Mix+S9	120	28.900	14.700
Air Part. + S9	120	0.089	0.019

Figure 5b - Exposure Time Experiment			
Air Part. +S9	2	0.080	0.007
Diesel+S9	2	2.410	0.370
PAH Mix +S9	2	10.580	1.510
Diesel No S9	2	10.600	2.970
PAH Mix No S9	2	5.870	0.217
Air Part. +S9	3	0.158	0.027
Diesel+S9	3	3.710	0.750
PAH Mix +S9	3	19.730	6.170
Diesel No S9	3	17.950	15.600
PAH Mix No S9	3	35.100	1.670
Air Part. +S9	4	0.140	0.037
Diesel+S9	4	3.490	0.633
PAH Mix +S9	4	38.420	13.970
Diesel No S9	4	5.560	1.800
PAH Mix No S9	4	16.250	4.165

5) Data used for Figure 6. Concentration-response data documenting the effect of chromogen incubation time density on the response of 4-NQO, MNNG, Diesel Particulate extract and PAH Mixture from Coal Tar.

Sample	Concentration (μg per assay mL)	Exposure Time (hrs)	Mean SOS Induction Factor	Standard Error
Mitomycin C	0.0310	2	1.815	0.058
Mitomycin C	0.0620	2	2.495	0.110
Mitomycin C	0.1250	2	3.570	0.144
Mitomycin C	0.2500	2	5.363	0.142
Mitomycin C	0.5000	2	8.464	0.345
Mitomycin C	1.0000	2	9.943	0.222
Mitomycin C	0.0310	3	3.979	0.007
Mitomycin C	0.0620	3	5.954	0.403
Mitomycin C	0.1250	3	9.462	0.642
Mitomycin C	0.2500	3	12.486	0.728
Mitomycin C	0.5000	3	14.594	0.554
Mitomycin C	1.0000	3	18.762	0.441
Mitomycin C	0.0310	4	5.471	0.525
Mitomycin C	0.0620	4	8.084	0.247
Mitomycin C	0.1250	4	11.043	0.680
Mitomycin C	0.2500	4	11.708	1.893
Mitomycin C	0.5000	4	15.987	2.093
Mitomycin C	1.0000	4	14.412	0.232
4-NQO	0.0390	2	2.626	0.020
4-NQO	0.0780	2	3.915	0.053
4-NQO	0.1560	2	7.946	0.230
4-NQO	0.3120	2	17.205	0.626
4-NQO	0.6250	2	25.194	0.533
4-NQO	1.2500	2	40.103	2.932
4-NQO	0.0390	3	1.802	0.102
4-NQO	0.0780	3	4.170	0.293
4-NQO	0.1560	3	9.617	0.715
4-NQO	0.3120	3	17.532	2.672
4-NQO	0.6250	3	27.670	2.997
4-NQO	1.2500	3	67.476	4.496
4-NQO	0.0390	4	1.494	0.029
4-NQO	0.0780	4	2.970	0.145
4-NQO	0.1560	4	6.495	0.368
4-NQO	0.3120	4	10.025	1.910
4-NQO	0.6250	4	24.177	2.710
4-NQO	1.2500	4	63.702	7.804
(mg per assay mL)				
Diesel Particulates	0.1950	2	8.319	0.160
Diesel Particulates	0.3910	2	12.610	0.992
Diesel Particulates	0.7810	2	17.744	0.759
Diesel Particulates	1.5620	2	18.878	1.921
Diesel Particulates	3.1250	2	13.913	1.125
Diesel Particulates	6.2500	2	4.086	0.202
Diesel Particulates	0.1950	3	10.679	0.878
Diesel Particulates	0.3910	3	21.567	1.948
Diesel Particulates	0.7810	3	28.752	1.465
Diesel Particulates	1.5620	3	79.029	8.455
Diesel Particulates	3.1250	3	45.891	5.635
Diesel Particulates	6.2500	3	9.255	0.330

Diesel Particulates	0.1950	4	8.591	0.768
Diesel Particulates	0.3910	4	12.912	1.197
Diesel Particulates	0.7810	4	15.395	3.030
Diesel Particulates	1.5620	4	20.940	1.115
Diesel Particulates	3.1250	4	36.434	14.324
Diesel Particulates	6.2500	4	15.595	1.761
PAH From Tar	0.00625	2	1.137	0.045
PAH From Tar	0.01250	2	1.598	0.066
PAH From Tar	0.02500	2	1.510	0.026
PAH From Tar	0.05000	2	1.612	0.112
PAH From Tar	0.10000	2	1.773	0.020
PAH From Tar	0.20000	2	1.243	0.045
PAH From Tar	0.00625	3	1.201	0.055
PAH From Tar	0.01250	3	1.804	0.121
PAH From Tar	0.02500	3	1.962	0.051
PAH From Tar	0.05000	3	2.016	0.039
PAH From Tar	0.10000	3	1.870	0.069
PAH From Tar	0.20000	3	1.570	0.042
PAH From Tar	0.00625	4	1.409	0.068
PAH From Tar	0.01250	4	1.530	0.029
PAH From Tar	0.02500	4	1.807	0.064
PAH From Tar	0.05000	4	1.852	0.070
PAH From Tar	0.10000	4	1.590	0.054
PAH From Tar	0.20000	4	1.162	0.073

6) Data used to generate, Figure 7.

Sample	Final S9 Conc. (% v/v)	Mean SRIP (IF per µg)	Std. Error
Pure Substances			
2-NA	0.75	1.465E-02	3.060E-03
Captan	0.75	8.227E-02	4.510E-03
NDMA	0.75	1.030E-04	1.670E-05
2-NA	1.50	1.700E-02	2.347E-03
Captan	1.50	7.545E-02	4.008E-03
NDMA	1.50	1.509E-04	2.849E-05
2-NA	3.00	2.920E-02	3.853E-03
Captan	3.00	4.460E-02	2.820E-03
NDMA	3.00	1.361E-04	1.497E-05
Complex Mixtures			
(mg sample Equiv.)			
Diesel Part.	0.5	5.450	0.885
Air Part.	0.5		
PAH Mix	0.5	7.500	0.875
Diesel Part.	1.0	3.680	0.379
Air Part.	1.0	0.140	0.029
PAH Mix	1.0	4.637	1.070
Diesel Part.	2.0	2.350	0.238
Air Part.	2.0	0.156	0.029
PAH Mix	2.0	12.410	1.490

Diesel Part.	4.0	1.330	0.103
Air Part.	4.0	0.075	0.008
PAH Mix	4.0	37.940	8.997

7) Historical summary of Mitomycin C and Benzo(a)pyrene concentration-response data used to generate top two panels of Figure 8.

Sample	Concentration (μg per assay mL)	Mean Induction Factor	Std. Error	2 x Std. Error
Benzo(a)pyrene	0.19531	1.3511	0.0254	0.0508
Benzo(a)pyrene	0.39063	1.9757	0.0467	0.0934
Benzo(a)pyrene	0.78125	2.8048	0.0598	0.1196
Benzo(a)pyrene	1.56250	3.1259	0.0570	0.1139
Benzo(a)pyrene	3.12500	3.0598	0.0478	0.0955
Benzo(a)pyrene	6.25000	3.2605	0.0638	0.1276
Mitomycin C	0.00625	1.2799	0.0188	0.0376
Mitomycin C	0.01250	1.6551	0.0298	0.0596
Mitomycin C	0.02500	2.3049	0.0478	0.0955
Mitomycin C	0.05000	3.5062	0.0764	0.1527
Mitomycin C	0.10000	5.9693	0.1496	0.2992
Mitomycin C	0.20000	12.0581	0.3796	0.7591

8) Data used to generate box plots in Figure 8. A- Measured SOS response inducing potency values for Benzo(a)pyrene and Mitomycin C.

Date	Plate #	SRIP (IF per μg per assay mL)
1) Benzo(a)pyrene		
DEC1	7	2.29071
DEC1	8	2.12716
DEC1	9	2.40870
DEC1	10	2.79400
DEC1	11	3.24087
DEC1	12	3.37210
MAR295	1	1.29912
MAR295	2	1.73969
MAR295	3	1.76122
MAR295	4	1.63125
MAR295	6	1.97900
MAR295	8	1.40003
MAR295	9	2.68033
MAR295	10	2.20553
MAR295	11	2.16759
MAR295	12	1.71153
NOV15	2	2.46390
NOV18	3	1.60180
NOV18	4	1.79218
NOV18	5	2.03037
NOV18	6	2.55469
NOV18	7	3.37313
NOV18	8	2.87047
NOV18	9	2.69722

NOV18	10	3.32797
NOV18	11	3.44198
NOV18	12	3.49418
NOV21	1	2.38056
NOV21	2	2.51504
NOV21	3	2.03142
NOV21	4	1.85497
NOV21	5	2.18769
NOV21	6	2.25831
NOV21	7	2.65381
NOV21	8	2.45823
NOV21	9	1.98790
NOV21	10	1.79988
NOV21	11	2.28364
NOV21	12	2.34594
NOV24	1	2.03377
NOV24	2	2.46545
NOV24	3	2.57865
NOV24	4	2.53539
NOV24	5	2.53147
NOV24	6	2.78927
NOV24	7	3.17629
NOV24	8	3.05325
NOV24	9	2.53355
NOV24	10	2.93303
NOV24	11	2.88258
NOV24	12	2.77844
NOV28	7	2.59893
NOV28	8	2.22231
NOV28	9	2.15257
NOV28	10	2.67328
NOV28	11	2.83383
NOV28	12	2.50548

2) Mitomycin C

DEC1	1	38.3375
DEC1	2	49.9062
DEC1	3	48.4452
DEC1	4	46.6511
DEC1	5	55.0794
DEC1	6	51.3136
MAR1095	1	36.6621
MAR1095	2	24.9463
MAR1095	3	32.8943
MAR1095	4	35.5497
MAR1095	5	51.3101
MAR1095	6	40.7912
MAR1095	7	30.1642
MAR1095	8	36.1461
MAR1095	9	17.5125
MAR1095	10	23.1588
MAR1095	11	27.3559
MAR1095	12	24.3315
MAR195	2	53.2047
MAR195	3	42.4618
MAR195	4	41.7067
MAR195	5	53.7807
MAR195	6	35.3192

MAR195	7	50.8934
MAR195	8	51.8191
MAR795	1	10.4141
MAR795	2	41.3032
MAR795	3	21.9283
MAR795	4	35.3479
MAR795	5	63.5683
MAR795	6	34.1123
MAR795	7	26.9398
MAR795	8	32.5450
MAR795	9	35.9815
MAR795	10	39.3970
MAR795	11	31.4435
MAR795	12	33.4591
MARCH11	1	79.9821
MARCH11	2	62.9494
MARCH11	3	51.4288
MARCH11	4	43.6148
MARCH11	5	29.9407
MARCH11	6	33.7536
MARCH11	7	30.3949
MARCH11	8	31.7214
MARCH11	9	29.4569
MARCH11	10	16.2299
MARCH11	11	21.0898
MARCH11	12	26.5444
MARCH14	1	32.0210
MARCH14	2	43.2930
MARCH14	3	40.7704
MARCH14	4	41.4500
MARCH14	5	30.4402
MARCH14	6	25.3622
MARCH14	7	36.3896
MARCH14	8	32.4367
MARCH14	9	25.1350
MARCH14	10	25.9003
MARCH14	11	32.0290
MARCH14	12	43.2121
MARCH7	1	46.6390
MARCH7	2	45.3804
MARCH7	3	53.8160
MARCH7	4	44.6206
MARCH7	5	57.9783
MARCH7	6	43.5037
MARCH7	7	39.3995
MARCH7	8	57.4415
MARCH7	9	51.8721
MARCH7	10	57.5683
MARCH7	11	46.8572
MARCH7	12	66.7662
MARCH9	1	48.1438
MARCH9	2	82.3375
MARCH9	3	90.9045
MARCH9	4	52.8381
MARCH9	5	29.1719
MARCH9	6	26.8580
MARCH9	7	28.6428
MARCH9	8	47.2659

MARCH9	9	29.6818
MARCH9	10	60.8690
MARCH9	11	60.8203
MARCH9	12	47.2378
MAY14	1	90.3524
MAY14	2	60.2887
MAY14	3	54.1719
MAY14	4	78.0628
MAY14	5	75.8267
MAY14	6	78.1853
MAY14	7	94.2557
MAY14	8	81.2866
MAY14	9	88.3163
MAY14	10	87.2352
MAY14	11	67.1290
MAY14	12	46.1630
MAY15	1	49.8790
MAY15	2	37.1890
MAY15	3	98.5630
MAY15	4	104.9150
MAY15	5	85.2540
MAY15	6	57.2360
MAY15	7	66.2790
MAY15	8	32.9550
MAY15	9	101.8340
MAY15	10	62.7920
MAY15	11	107.4370
MAY15	12	76.2980
NOV15	1	49.5420
NOV17	1	53.7060
NOV17	2	44.9620
NOV17	3	53.4780
NOV17	4	52.5170
NOV17	5	80.9420
NOV17	6	66.9050
NOV17	7	74.6760
NOV17	8	77.1560
NOV17	10	77.3400
NOV17	11	68.5450
NOV17	12	77.4660
NOV22	1	50.2300
NOV22	2	56.1060
NOV22	3	50.1620
NOV22	4	62.4080
NOV22	5	44.2860
NOV22	6	44.2530
NOV22	7	41.8940
NOV22	8	37.7450
NOV22	9	23.8260
NOV22	10	35.1470
NOV22	11	29.5240
NOV22	12	33.7630
NOV25	1	48.6620
NOV25	2	45.5420
NOV25	3	46.5790
NOV25	4	56.2840
NOV25	5	47.7980
NOV25	6	57.6760

NOV25	7	47.7579
NOV25	8	43.6329
NOV25	9	69.5759
NOV25	10	47.5219
NOV25	11	49.5679
NOV25	12	66.1616
NOV28	2	56.9652
NOV28	3	50.3765
NOV28	4	54.7392
NOV28	5	48.6359
NOV28	6	46.4329

9) Literature data used to generate box plots in Figure 8. SOS Response Inducing potency for Benzo(a)pyrene and Mitomycin C. All potency values in IF per μg per assay mL.

Author	Year	SOSIP	MaxIF	S9 Conc % v/v	Procedure
1) Benzo(a)pyrene					
Mersch-Sundermann et al	1992	1.170	6.84	4.5%	Standard with modifications
Von der Hude et al	1988	0.205	5.34	9.0%	Standard
Quillardet	unpub	5.770	8.10	prob 9%	Standard
Venier et al	1989	0.795	9.42	9.0%	Standard
Venier et al	1989	1.800	9.61	9.0%	Standard
Mersch-Sundermann	1993	0.690	4.26	9.0%	Standard with modifications
Mersch-Sundermann	1993	1.500	5.83	4.5%	Standard with modifications
Schleibinger et al	1989	1.810	3.60	???	German text
Tudek et al	1988	0.218	8.64	9.0%	Standard
Marzin et al	1986	0.305	2.93	6.0%	Kinetic Method
Rempola et al	1986	2.770	3.24	3.6%	Standard
	Mean	1.55	6.16		
	Std. Error	0.49	0.76		
2) Mitomycin C					
Auffray and Boutibonnes	1987	75.00	7.00	Not applicable	Standard
Quillardet	unpub	209.80	15.40	"	Standard
Ohta et al	1984	85.26	7.40	"	Standard with modifications
Venier et al	1989	61.74	16.14	"	Standard
Rempola et al	1986	94.25	3.97	"	Standard
	Mean	105.21	9.98		
	Std. Error	26.70	2.44		

10) Data used for Figure 9. Standard error and upper confidence limit on mean induction factor value for solvent controls. Standard error values calculated according to Welsch et al. (1988). Upper confidence limit values are sorted in ascending order.

Date of Assay	Plate #	Standard Error of Mean Ratio	Upper 95% Confidence Limit
MAR295	2	0.00483	1.00829
NOV24	1	0.00596	1.01024
MAR295	4	0.00738	1.01266
NOV18	2	0.00805	1.01383
MAR295	3	0.00818	1.01405
NOV18	6	0.00821	1.01409
NOV21	2	0.00848	1.01456
NOV24	2	0.00852	1.01463
NOV24	4	0.00865	1.01485
NOV18	8	0.00912	1.01565
NOV18	7	0.00916	1.01574
NOV18	3	0.00954	1.01638
NOV18	5	0.00974	1.01672
NOV24	3	0.00986	1.01693
NOV18	4	0.01053	1.01808
MAR295	1	0.01068	1.01834
NOV21	1	0.01084	1.01861
NOV17	3	0.0113	1.01941
JULY16	11	0.01098	1.01990
MAR195	6	0.01167	1.02003
MAR795	7	0.01242	1.02133
AUG2	1	0.01179	1.02136
JULY16	3	0.01184	1.02145
DEC1	12	0.01289	1.02214
NOV18	12	0.01295	1.02223
DEC1	11	0.01296	1.02226
JULY16	9	0.01258	1.02279
DEC1	1	0.01377	1.02365
NOV24	8	0.01379	1.02368
DEC1	2	0.01385	1.02378
NOV18	11	0.01407	1.02416
JULY14	9	0.01334	1.02418
JULY16	10	0.01363	1.02470
NOV17	5	0.01445	1.02482
JULY10	6	0.01397	1.02531
JULY19	11	0.01403	1.02543
NOV17	2	0.01485	1.02549
NOV21	3	0.01490	1.02559
JULY10	3	0.01433	1.02596
JULY16	12	0.01433	1.02597
NOV17	10	0.01579	1.02711
JULY16	4	0.01522	1.02758
NOV18	1	0.01610	1.02764
NOV24	6	0.01613	1.02769
NOV21	4	0.01634	1.02806
NOV17	4	0.01655	1.02841
JULY16	7	0.01589	1.02880
AUG2	5	0.01589	1.02880
DEC1	3	0.01678	1.02882
JULY19	7	0.01592	1.02884
JULY16	2	0.01601	1.02901
JULY10	5	0.01602	1.02904
MAR295	8	0.01700	1.02918

NOV15	2	0.01723	1.02958
MAR295	6	0.01728	1.02967
MAR795	8	0.01747	1.02999
NOV17	1	0.01761	1.03024
NOV17	6	0.01765	1.03031
JULY19	12	0.01685	1.03053
NOV17	7	0.01863	1.03198
NOV24	7	0.01873	1.03217
JULY19	8	0.01827	1.03311
AUG2	6	0.01832	1.03319
DEC1	8	0.01950	1.03349
AUG2	12	0.01864	1.03378
NOV17	11	0.01986	1.03411
JULY10	4	0.01887	1.03419
DEC1	4	0.02007	1.03446
MAR11	6	0.01945	1.03524
MAR11	5	0.01957	1.03546
NOV17	12	0.02099	1.03604
NOV18	10	0.02165	1.03717
MAR295	7	0.02182	1.03747
JULY10	7	0.02155	1.03906
MAR11	4	0.02160	1.03914
NOV22	1	0.02286	1.03924
JULY16	6	0.02192	1.03972
AUG3	8	0.02193	1.03973
AUG3	10	0.02210	1.04004
NOV17	8	0.02347	1.04030
MAR11	1	0.02229	1.04039
AUG3	1	0.02314	1.04194
JULY16	1	0.02323	1.04210
JULY14	11	0.02327	1.04217
DEC1	10	0.02461	1.04225
NOV24	5	0.02471	1.04243
MAR795	11	0.02522	1.04331
NOV24	12	0.02529	1.04343
AUG2	8	0.02398	1.04345
JULY14	12	0.02422	1.04388
AUG2	9	0.02429	1.04400
AUG3	6	0.02436	1.04413
MAR1095	12	0.02580	1.04429
MAR795	1	0.02628	1.04512
AUG1	10	0.02505	1.04538
NOV22	3	0.02646	1.04544
MAR7	10	0.02533	1.04590
AUG3	9	0.02566	1.04649
AUG3	5	0.02578	1.04672
JULY10	11	0.02610	1.04730
AUG2	3	0.02614	1.04737
JULY16	8	0.02614	1.04737
MAR9	8	0.02678	1.04853
MAR1095	1	0.02855	1.04902
JULY10	12	0.02715	1.04919
JULY10	10	0.02717	1.04923
NOV21	11	0.02906	1.04990
JULY14	7	0.02760	1.05001
AUG3	2	0.02810	1.05091
NOV21	6	0.02971	1.05101
NOV24	11	0.03014	1.05176

MAR7	6	0.02862	1.05186
AUG2	7	0.02867	1.05196
AUG2	10	0.02896	1.05248
MAR7	9	0.02905	1.05263
AUG3	7	0.02923	1.05297
MAR7	3	0.02924	1.05298
JULY19	6	0.02943	1.05332
JULY9	7	0.02946	1.05338
MAR9	10	0.02949	1.05343
JULY19	9	0.02976	1.05392
NOV21	12	0.03152	1.05411
JULY10	1	0.02993	1.05423
MAR1095	10	0.03160	1.05425
MAY14	7	0.03160	1.05425
JULY10	2	0.03006	1.05446
MAR295	12	0.03176	1.05454
JULY19	10	0.03019	1.05471
MAR11	3	0.03034	1.05498
MAR1095	8	0.03205	1.05502
DEC1	7	0.03231	1.05547
NOV22	2	0.03249	1.05579
NOV15	1	0.03277	1.05627
JULY19	5	0.03118	1.05650
JULY10	9	0.03127	1.05667
DEC1	5	0.03302	1.05669
NOV25	1	0.03316	1.05693
NOV21	8	0.03320	1.05701
AUG1	9	0.03148	1.05705
MAY15	2	0.03342	1.05738
NOV22	4	0.03371	1.05788
MAR795	3	0.03375	1.05794
NOV21	7	0.03420	1.05873
JULY31	3	0.03247	1.05883
MAR7	5	0.03268	1.05922
JULY9	5	0.03287	1.05956
MAR1095	5	0.03535	1.06070
MAR195	5	0.03547	1.06089
JULY14	10	0.03376	1.06118
JULY16	5	0.03381	1.06127
MAY14	1	0.03570	1.06130
MAR11	11	0.03394	1.06150
MAR9	12	0.03401	1.06163
MAY14	12	0.03596	1.06173
JULY14	6	0.03407	1.06174
AUG2	11	0.03412	1.06182
NOV21	5	0.03625	1.06223
DEC1	6	0.03629	1.06231
JULY14	3	0.03443	1.06238
JULY31	7	0.03455	1.06261
JULY31	8	0.03497	1.06337
MAY15	7	0.03701	1.06355
MAR795	2	0.03717	1.06382
AUG2	4	0.03559	1.06450
NOV21	10	0.03761	1.06458
MAY14	4	0.03765	1.06465
JULY14	4	0.03619	1.06558
MAY14	2	0.03831	1.06578
MAR295	11	0.03842	1.06597

JULY9	4	0.03665	1.06641
DEC1	9	0.03868	1.06641
AUG1	3	0.03694	1.06693
MAY15	6	0.03898	1.06694
JULY9	3	0.03716	1.06733
MAR195	4	0.03937	1.06760
MAR1095	4	0.03954	1.06790
MAY14	8	0.03970	1.06817
JULY19	4	0.03791	1.06870
JULY9	8	0.03806	1.06897
AUG3	3	0.03863	1.07000
MAR11	12	0.03875	1.07022
MAR1095	9	0.04106	1.07051
NOV24	10	0.04127	1.07085
MAR11	10	0.03913	1.07089
AUG1	5	0.03924	1.07111
MAR11	9	0.03939	1.07137
MAY14	5	0.04191	1.07196
MAY14	10	0.04196	1.07204
MAR11	7	0.03987	1.07225
MAR1095	11	0.04222	1.07249
JULY14	2	0.04047	1.07333
MAY14	11	0.04296	1.07377
JULY31	12	0.04090	1.07411
MAY14	3	0.04324	1.07424
MAR1095	3	0.04335	1.07443
AUG1	7	0.04128	1.07481
AUG1	2	0.04129	1.07481
MAR795	10	0.04365	1.07494
AUG1	8	0.04188	1.07589
MAR7	12	0.04215	1.07637
AUG1	4	0.04228	1.07662
AUG3	4	0.04241	1.07684
JULY19	3	0.04248	1.07698
AUG2	2	0.04260	1.07718
MAR7	7	0.04260	1.07720
MAR1095	2	0.04513	1.07748
MAR795	5	0.04517	1.07756
MAY14	6	0.04544	1.07801
MAR9	5	0.04328	1.07843
MAR795	9	0.04599	1.07897
MAY15	11	0.04606	1.07908
MAR7	11	0.04372	1.07921
MAR9	2	0.04437	1.08040
NOV24	9	0.04708	1.08084
NOV25	2	0.04754	1.08162
NOV21	9	0.04765	1.08181
MAR1095	7	0.04767	1.08184
NOV22	6	0.04767	1.08186
MAY14	9	0.04776	1.08201
MAR295	9	0.04801	1.08243
MAY15	8	0.04855	1.08336
JULY31	9	0.04618	1.08368
JULY9	2	0.04626	1.08382
JULY9	10	0.04643	1.08413
MAR9	6	0.04645	1.08416
MAR1095	6	0.04903	1.08418
MAR7	8	0.04655	1.08435

MAY15	3	0.04916	1.08440
JULY9	6	0.04681	1.08483
AUG1	1	0.04682	1.08483
JULY19	2	0.04709	1.08533
JULY19	1	0.04718	1.08548
MAR795	4	0.04980	1.08550
MAR9	11	0.04738	1.08586
JULY10	8	0.04777	1.08657
MAR7	1	0.04835	1.08760
NOV22	8	0.05110	1.08774
MAY15	9	0.05132	1.08812
JULY31	4	0.04866	1.08817
JULY9	11	0.04867	1.08819
NOV25	4	0.05158	1.08857
MAR9	9	0.04918	1.08911
MAR195	7	0.05334	1.09158
JULY31	10	0.05071	1.09188
MAR9	4	0.05106	1.09252
MAR9	7	0.05120	1.09278
MAR7	4	0.05122	1.09281
JULY31	6	0.05125	1.09286
JULY9	1	0.05142	1.09317
AUG1	6	0.05151	1.09334
MAY15	12	0.05497	1.09438
MAY15	5	0.05703	1.09793
MAR9	3	0.05410	1.09803
MAY15	10	0.05764	1.09896
MAY15	4	0.05791	1.09943
MAY15	1	0.05807	1.09971
JULY14	8	0.05573	1.10098
JULY31	1	0.05593	1.10134
MAR795	12	0.06036	1.10364
JULY9	12	0.05721	1.10366
NOV25	8	0.06075	1.10431
MAR11	2	0.05765	1.10447
JULY14	1	0.05798	1.10505
MAR795	6	0.06129	1.10523
MAR11	8	0.05872	1.10640
JULY14	5	0.05980	1.10835
NOV22	7	0.06322	1.10855
NOV22	12	0.06336	1.10880
MAR195	8	0.06423	1.11029
JULY31	11	0.06135	1.11117
NOV22	9	0.06507	1.11172
NOV25	7	0.06666	1.11445
NOV22	5	0.06707	1.11515
JULY9	9	0.06457	1.11699
NOV25	6	0.07319	1.12567
NOV25	3	0.07342	1.12606
JULY31	2	0.07266	1.13166
NOV22	11	0.07953	1.13656
NOV22	10	0.08003	1.13742
MAR195	2	0.08306	1.14262
NOV17	9	0.09028	1.15502
MAR7	2	0.08772	1.15895
MAR195	3	0.09568	1.16428
JULY31	5	0.09632	1.17452
MAR295	10	0.10341	1.17755

NOV25	5	0.10653	1.18292
MAR9	1	0.10158	1.18407
NOV25	10	0.11562	1.19851
NOV25	9	0.11717	1.20118
NOV18	9	0.12503	1.21467
NOV25	11	0.13111	1.22511
MAR295	5	0.15865	1.27240
NOV25	12	0.17583	1.30190

APPENDIX 4

Genotoxic, mutagenic and carcinogenic potency data used to generate models and figures in Chapter 2.

1. Genotoxic and Mutagenic Potency Data

All values are arithmetic means of raw data collected from the literature. Numbers in brackets denote sample size. Where not indicated, samples size was 1. SOS Chromotest genotoxic potency values are expressed in Induction factor units per µg of sample per assay ml. Data from the literature were standardized to an assay volume of 1.0 ml. Where the assay volume was not indicated, the volume recommended by Quillardet and Hofnung (1985) was assumed (ie. 0.62 ml.). Unless otherwise indicated, SOS genotoxicity was measured using *Escherichia coli* PQ37. [†] Indicates that *E. coli* PQ30 (not *arrA* or *rfa*) was used. Mutagenic potency values are expressed as his Revertents per µg of sample per assay ml. Data from the literature were standardized to an assay volume of 1.0 ml. Where the assay volume was not indicated, the volume recommended by Maron and Ames (1983) was assumed (ie. 2.2 ml. without S9 and 2.7 ml. with S9). In most cases Ames mutagenic potency values were determined using the standard plate incorporation assay (Ames *et al.*, 1975; Maron and Ames, 1983). * Indicates that the pre-incubation method of Yahagi *et al.*, (1975) was employed.

A- Direct acting substances.

Compound Name	SOS Chromotest Genotoxic Potency (IF per µg per ml.)				Ames Test Mutagenic Potency (his revertents per µg per ml.)			
	SOSIP	References	TA100	References	TA98	References	Other Strains	References
1,2-Dibromoethane	3.38E-03 (3)	1,2,3	6.70E-01 (3)	4,5,6				
1,2-Epoxy-3,3,3-trichloropropene	2.79E-02	7	5.31E+00	7				
1,2-Epoxy-3-butene	1.21E-04	7	1.80E-01	7			TA1535: 3.88E-01	7
1,2-Epoxybenzene	6.63E-05	7	3.75E-01	7				
1,2-bis(2,3-epoxypropoxy)butane	7.23E-03	7	5.84E-01	7				
1,2-bis(2,3-epoxypropoxy)ethane	6.82E-03	7	9.99E-01	7			TA1535: 9.99E-01	7
1,2,3,4-Diepoxybutane	6.66E-02 (2)	2,7	1.39E+00 (2)	8,7			TA1535: 3.07E+00	4
1,2,3,6-Diepoxyhexane	8.37E-04	7	2.16E+00	7			TA1535: 1.89E+01	7
1,2,7,8-Diepoxyoctane	6.86E-03	7	1.02E+00 (2)	7,4	3.08E-02	9	TA97: 3.48E-01	9
1,3-Dichloro-2-propyl-methane sulphonate	5.61E-03	10	5.10E-02	10 [†]				
1,3-Dinitropyrene	3.20E+02	11	6.29E+04	12	3.11E+05 (2)	12,13	TA1537: 1.01E+05	12
							TA1538: 1.17E+05	12
1,3-Propene Sulfone	1.15E-01 (2)	1,14	5.04E+01	15			TA1535: 1.19E+02	4
1,6-Dinitropyrene	8.83E+02	11			2.74E+05	12	TA1537: 2.48E+05	12
							TA1538: 9.11E+04	12
1,8-Dinitropyrene	7.38E+02	11			1.01E+06 (2)	12,13	TA1537: 8.88E+04	12
							TA1538: 7.49E+04	12
1-Nitronaphthalene	7.32E-02	11	1.10E+1 (2)	16,17	1.69E+00 (2)	16,17		
1-Nitropyrene	6.70E+00 (3)	11,18,3	4.89E+02 (2)	18,12	5.01E+03 (2)	18,12	TA1537: 5.96E+02	12
							TA1538: 7.63E+02 (2)	18,12
1-Phenyl-2-butyl-methane sulphonate	1.03E-01	10	6.36E+00	10 [†]				
2,2-Dimethylpropyl-methanesulphonate	7.92E-03	10	5.27E-01	10 [†]				
2,3-Dichloropropene	5.36E-03	2	6.38E+01	19**			TA1535: 1.86E+01	19
2,3-Epoxy-1-propanol (Glycidol)	2.88E-03	7	7.22E-01	7			TA1535: 1.72E+01	4
2,3-Epoxypropyl phenylether	9.44E-03	7	5.58E+00	7				
2,4,5,7-Tetrachloro-9-fluorenone	7.36E+00	11	1.75E+03	20	5.25E+03	20 [†]	TA1535: 3.05E+01	20
							TA1537: 1.14E+03	20
							TA1538: 1.34E+04	20
2,4,7-Trinitro-9-fluorenone	6.40E+00 (2)	11,21	2.74E+03 (2)	20,22	4.43E+04 (4)	43 [†] ,20,24 [†] ,22	TA102: 1.76E+02	22
							TA97: 1.25E+05	22
							TA1537: 3.99E+03	20
							TA1538: 2.00E+04	20
2,4,7-Trinitro-phenanthrene-quinone	5.12E+00 (2)	25,26	1.94E+03	26			TA1538: 1.91E+03	25

2,4-Dinitrophenylthiocyanate	9.09E-02	3	5.26E+02	6					
2,7-Dinitro-9-fluorenone	2.17E+00 (2)	11,18	3.62E+03 (2)	18,20		3.01E+04 (2)	18,20	TA1537: 1.59E+03 (2)	20
2,7-Dinitro-9-fluorenone-4-carboxamide	1.28E+00 (2)	25,26				5.08E+02 (2)	25,26	TA1538: 3.22E+04 (2)	18,20
2,7-Dinitrofluorene	7.71E+00	11	5.07E+01	20		1.30E+04 (2)	20,13	TA1538: 4.20E+03 (2)	25,26
2,7-Dinitrophenanthrenequinone	4.65E+00 (2)	25,26				3.44E+02 (2)	25,26	TA1537: 2.49E+03	20
2-Amino-5-nitrothiazole	1.92E-01	2	3.37E-01 (2)	27				TA1538: 2.97E+03	20
2-Bromocrocin	5.67E+02	23	2.37E+04	23 ⁶				TA1538: 2.80E+03 (2)	25,26
2-Butyl-methanesulphonate	1.12E-01	10	1.99E+01	10 ⁶					
(Z)-2-Chloro-3-(dichloromethyl)-4-oxobutenoic acid	2.80E+02	40	6.29E+04	40		1.96E+03	40	TA97: 1.05E+04	40
(Z)-2-Chloro-3-(dichloromethyl)-4-hydroxybut-2-enoic acid	5.32E+00	40	8.02E+02	40		1.21E+01	40	TA97: 4.01E+02	40
(E)-2-Chloro-3-(dichloromethyl)-4-oxobutenoic acid ¹¹	1.95E+01	40	3.13E+03	40		1.31E+02	40		
2-Chloro-3,3-dimethylacrolein	2.01E+00	23	8.23E+02	23 ⁶					
2-Chloroacrolein	3.74E+02	23	4.31E+04	23 ⁶					
2-Chloroethyl-methanesulphonate	5.98E-03	10	7.07E-01	10 ⁶					
2-Methylpropyl-methanesulphonate	7.24E-03	10	3.77E-01	10 ⁶					
2-Nitro-5-methoxy-benzofuran (R5255)	3.23E+02 (2)	1,28	7.18E+03	15		6.27E+02	28	TA104: 2.33E+06	28
2-Nitro-7-methoxy-naphthofuran (R7000)	5.25E+04 (4)	1,28,29,30	1.71E+06 (3)	31,15,28		6.65E+05 (3)	3,28,29	TA97: 4.36E+05	3
								TA1537: 4.36E+05	28
2-Nitro-8-methoxy-naphthofuran (R6598)	4.76E+04	1	1.65E+06	15					
2-Nitro-11-methoxy-naphthofuran (R7100)	2.72E+04	1	4.07E+05	15		1.36E+05	28		
2-Nitro-naphthofuran (R6597)	2.56E+04 (2)	1,28	2.08E+05	15		2.18E+04	28		
2-Nitronaphthofuran Derivative R6998	4.44E+04	28	2.17E+06 (2)	28		5.07E+05	28		
2-Nitronaphthofuran Derivative R7105	2.27E+03	28	9.96E+03	28		2.44E+04	28		
2-Nitronaphthofuran Derivative R7134	2.25E+03	28	8.00E+04	28		5.87E+04	28		
2-Nitronaphthofuran Derivative R7160	9.04E+04	28	9.79E+04	28		3.01E+04	28		
2-Nitronaphthofuran Derivative R7202	6.91E+03	28	1.63E+05	28		1.63E+04	28		
2-Nitronaphthofuran Derivative R7216	1.18E-02	120	9.23E-01	28					
2-Nitronaphthofuran Derivative R7220	1.78E+04	28	3.44E+04	28		1.63E+04	28		
2-Nitronaphthofuran Derivative R7221	2.26E+03	28	3.47E+03	28		1.51E+04	28		
2-Nitronaphthofuran Derivative R7237	1.73E-02	28	6.88E-01	28		3.48E-02	28		
2-Nitronaphthofuran Derivative R7325	3.32E+02	28	6.49E+03	28		1.32E+03	28		
2-Nitronaphthofuran Derivative R7333	4.98E+02	28	1.32E+04	28		4.36E+03	28		
2-Nitronaphthofuran Derivative R7334	2.35E+02	28	9.02E+03	28		3.95E+03	28		
2-Nitronaphthofuran Derivative R7355	4.16E+01	28	2.24E+03	28		3.23E+02	28		
2-Nitronaphthofuran Derivative R7368	6.75E+03	28	3.33E+05	28		2.00E+04	28		
2-Nitronaphthofuran Derivative R7369	4.94E+03	28	2.29E+05	28		2.76E+03	28		
2-Nitronaphthofuran Derivative R7370	3.38E+02	28	5.81E+04	28		1.14E+04	28		
2-Nitronaphthofuran Derivative R7371	2.14E+05	28	4.94E+07	28		7.85E+05	28		
2-Nitronaphthofuran Derivative R7372	1.17E+05	28	1.63E+07	28		1.11E+06	28		
2-Nitronaphthofuran Derivative R7419	7.68E-02	28	1.20E+02	28					
2-Nitronaphthofuran Derivative R7427	5.23E+04	28				1.37E+05	28		
2-Nitronaphthofuran Derivative R7489	1.09E+04	30				4.83E+05	30		
2-Nitronaphthofuran Derivative R7490	3.72E-02	30				1.45E+00	30		
2-Nitronaphthofuran Derivative R7516	1.40E+04	28				3.34E+04	28		
2-Nitronaphthofuran Derivative R7543	5.42E+01	28	6.21E+04	28					
2-Nitronaphthofuran Derivative R7615	5.51E+02	28	3.72E+04	28					
2-Nitronaphthofuran Derivative R7686	1.78E+05	29				7.95E+05	29		
2-Nitronaphthofuran Derivative R7688	2.89E+04	29				6.63E+04	29		
2-Nitronaphthofuran Derivative R7691	2.09E+05	30				2.84E+06	30		
2-Nitronaphthofuran Derivative R7707	1.34E+05	29				6.08E+05	29		
2-Nitrobenzofuran (R5144)	1.23E+02 (2)	1,28	4.72E+03	15		2.70E+02	28		

2-Nitronaphthalene	1.87E-01 (2)	17	5.49E+01 (5)	16,17,8,4	3.25E+00 (2)	11,17	TA1535: 1.78E+01 TA1537: 2.54E-01	16 16
2-Phenylethyl-methanesulphonate	3.82E-02	10	3.69E+00	10 ⁶				
2-Phenylpropyl-methanesulphonate	4.43E-03	10	3.55E+00	10 ⁶				
2-Propyl-methanesulphonate	2.70E-01	10	4.08E+01	10 ⁶				
2-Nitrofluorene	3.50E-01 (5)	1,14,11,32	1.45E+02 (3)	18,20,33	3.27E+02 (7)	28,11,18,20,33,34,13	TA97: 9.81E+01 TA1535: 1.04E-01 TA1537: 2.63E+00 (2) TA1538: 2.21E+02 (4) TA1538: 7.36E+02 (2)	9 33 20,9 4,18,20,9 25,26
2-Nitro-phenanthrene-quinone	2.26E+00 (2)	27						
3-(5-nitro-2-furyl)-acrylamide	8.43E+01	35	7.52E+02	36	1.41E+01	37 ⁶	TA1535: 4.11E+00	36
3,3-Dimethyl-2-butyl-methanesulphonate	7.98E-03	10	5.29E-01	10 ⁶				
3,3-Dimethylacrolein	1.75E-02	23	2.42E+00	23 ⁶				
3-Chloro-3-methylacrolein	2.04E-02	23	3.80E+00	23 ⁶				
3-Methyl-2-butyl-methanesulphonate	1.36E-02	10	1.19E+00	10 ⁶				
3-Nitro-9-fluorenone	2.31E+00	11			2.72E+03 (2)	11,13		
3-Nitrofluoranthene	4.97E+01 (2)	11,18	1.45E+04	18	3.14E+04 (3)	11,18,13	TA1538: 1.96E+04	18
3-Pentyl-methanesulphonate	3.90E-02	10	4.87E+00	10 ⁶				
4-Hydroxylaminoquinoline-N-oxide	2.04E+00	3	4.41E+02 (3)	8,38,4	1.14E+02	38	TA1535: 2.11E+01 TA1537: 1.33E+01 TA1538: 8.59E+01	38 38 38
4-Nitro-o-phenylenediamine	1.97E-02	39	7.17E+01 (2)	39,22	2.94E+02 (2)	39,22	TA97: 1.57E+02 (2) TA1537: 3.70E+00 TA1538: 1.67E+02	9,22 9 9
4-Nitroquinoline-N-oxide	1.16E+02 (30)	1,2,40,41,42,14,43,11,44,45,46,18,24,47,48,21,50,51,52,32,53,54,49,55,56,57,58,30,59	2.25E+04 (8)	15,4,28,22,60,33,61,62	5.54E+03 (8)	9,40,28,22,60,8,33,62	TA102: 1.26E+03 TA97: 3.19E+03 (2) TA1535: 1.39E+03 TA1537: 1.76E+01	22 9,22 33 9
5-Aziridino-2,4-dinitrobenzamide (CB1954)	4.88E-02	76	2.35E+02	60	1.06E+01	60		
5-Nitro-1-naphthnitrile	1.15E-01	3	1.04E+02	6			TA1535: 2.90E+00 TA1538: 7.50E+01	63 63
5-Nitro-2-furfural	1.65E+00	43	1.51E+02	43 ⁶				
5-Nitro-2-furylacrolein	4.77E+01	43	9.13E+03	43 ⁶				
5-Nitro-2-furylacrylic acid	1.67E+01 (4)	35,64,65,20	8.07E+02	36	2.58E+00	37 ⁶	TA1535: 5.87E+01 TA1538: 1.56E+01 (2)	36 25,26
7-Nitro-9-fluorenone-4-carboxylic acid	7.79E-01 (2)	25,26						
8-Methoxypsoralen	6.02E-03	143			1.03E+00	146		
2-Propenal (Acrolein)	3.71E-02	23	1.12E+02	23 ⁶			TA104: 4.27E+01 TA97: 1.98E+03 TA1537: 1.43E+03	66 ⁶ 8 4
Acridine ICR170	2.73E+00 (2)	2,3					TA97: 3.47E+03 (5) TA1537: 1.76E+03 TA1538: 1.48E+02	9,22,94,8 4,94,9 9
Acridine ICR191	2.98E+00 (2)	2,93	4.07E+02	22	1.39E+02	22	TA1538: 1.55E+01	69
Adriamycin (Doxorubicin)	4.00E+00 (2)	67			3.71E+02 (3)	63,68,4		
Albinoxin B1	2.01E-01	70	2.59E+02 (2)	71,72	1.76E+01	71		
Allyl-methanesulphonate	1.01E-01	10	4.20E+01	10 ⁶				
Aniline	4.51E-01	37					TA1535: 1.26E+01	38 ⁶
Ascorbic acid	2.72E+00 (2)	1,3	1.52E+04	1				
Baldriol	1.00E+02 (2)	2,34	1.68E+01	34	1.75E+00	34		
Benz(a)pyrene-7,8-diol-9,10-oxide(aaa)	2.18E+02	73	1.09E+05	73	2.69E+04	73	TA104: 1.09E+05 TA97: 3.66E+04	73 73
Benz(a)pyrene-7,8-diol-9,10-oxide(rya)	1.83E+02	73	2.10E+05	73	6.43E+04	73	TA104: 9.81E+04 TA97: 1.69E+05	73 73
Benz(a)chrysen-9,10-diol-11,12-oxide(aaa)	4.33E+02	73	4.11E+04	73	3.68E+04	73	TA104: 8.02E+04 TA97: 1.33E+05	73 73

Benzo(c)chrysene-9,10-diol-11,12-oxide(rya)	2.78E+02	73	3.27E+04	73	1.26E+04	73	TA104: 4.71E+04	73
Benzo(c)phenanthrene-3,4-diol-1,2-oxide(aui)	2.84E+02	73	1.63E+04	73	3.36E+03	73	TA97: 4.98E+04	73
Benzo(c)phenanthrene-3,4-diol-1,2-oxide(rya)	1.52E+02	73	1.38E+04	73	3.94E+03	73	TA104: 3.11E+04	73
Benzo(g)chrysene-11,12-diol-13,14-oxide(aui)	2.04E+02	73	2.51E+04	73	2.66E+03	73	TA97: 2.13E+04	73
Benzo(g)chrysene-11,12-diol-13,14-oxide(rya)	6.00E+01	73	8.87E+03	73	6.86E+02	73	TA104: 1.87E+04	73
Bleomycin	1.45E+02 (4)	1,14,3,32					TA97: 1.15E+04	73
1-Buthydropoxide	1.68E-01 (2)	2,59	5.10E+00	103			TA104: 2.92E+04	73
							TA97: 2.56E+04	73
							TA104: 1.71E+04	73
							TA97: 1.36E+04	73
							TA102: 2.34E+03 (2)	74,75
							TA102: 5.54E+01 (2)	74,75
							TA104: 1.32E+02	103
Captan	1.41E+01 (3)	47,3,77	3.86E+02 (5)	4,6,77,8,78			TA1535: 2.90E+01 (2)	79,78
Captafol	1.05E+01	3					TA1538: 1.82E+00 (2)	79,78
Carbadox	4.15E+01	3	9.13E+01 (2)	80,81	1.19E+02	80	TA1535: 2.20E-01	79
Ciprobaxacin	2.76E+02	82					TA102: 7.29E+04	82
Chrysene-1,2-diol-3,4-oxide(aui)	5.69E+01	73	5.41E+03	73	7.73E+02	73	TA104: 5.56E+03	73
Chrysene-1,2-diol-3,4-oxide(rya)	1.04E+01	73	1.55E+03	73	1.55E+02	73	TA97: 7.42E+02	73
							TA104: 2.94E+03	73
							TA97: 7.42E+02	73
Cyclohexyl-methanesulphonate	2.39E-02	10	1.84E+00	10 ⁶				
Cumene hydroperoxide	2.03E-01 (2)	2,59					TA102: 2.63E+01 (2)	74,75
							TA104: 7.53E+01	74
Dacarbazine	2.99E+00 (2)	67	1.95E+00	83	1.92E-01	83	TA97: 1.31E+01	83
Dactinomycin (Daunorubicin)	1.71E+01 (3)	1,3,21	1.72E+01	22	1.28E+03 (5)	4,22,63,68,13	TA102: 2.17E+02	22
							TA97: 4.55E+01	83
							TA1538: 3.11E+01	84
Sodium p-dimethylaminobenzene diazonium salt (Decon)	1.82E-01	3	8.05E+00	22	5.27E+01 (2)	22,9	TA102: 3.94E+01	22
							TA97: 1.18E+02 (2)	22
							TA1537: 4.58E+01	79
							TA1538: 1.80E+01	79
Diesel Particulate Matter (SRM 1650) TM	1.57E-02 (2)	85	8.29E+00	86	6.15E+00	86	TA97: 2.19E+00	9
Diethyl sulphate	1.73E-02 (2)	1,2	7.13E-01	15			TA1537: 2.77E-02	9
1,3-Dihydroxy-2-propanone (Dihydroxyacetone)	2.61E-04	2					TA104: 1.39E-01	66 ⁶
Dimethyl carbamoyl chloride	1.12E-02	1	6.92E-01	87	4.33E-02	87	TA1535: 9.12E-01 (3)	38 ⁶ , 87,4
							TA1537: 4.26E-02	87
Dimethyl sulphate	1.90E-01	1	1.74E+00	15				
Enoxacin	4.21E+01	82					TA102: 8.00E+03	82
Epichlorohydrin	3.24E-03	7	1.43E+00	7				
Epichlorohydrin	2.99E-02 (5)	2,14,48,21,7	3.29E+00 (4)	8,7,88,89			TA1535: 4.41E+00	89
Epichlorohydrin	6.51E-04	7	9.15E-01	7				
Ethyl-methanesulphonate	3.12E-03 (5)	1,2,14,10,3	1.14E+00 (3)	15,4,10 ⁶				
Ethyl vinyl ketone	1.90E-01	41	3.38E+01	41				
Ferric dimethyldithiocarbamate (Ferbam)	5.66E-01	47	7.03E+00	47				
N-(trichloromethylmercapto)-phthalimide (Polpet)	1.40E+01	47	6.80E+01 (3)	6,8,78			TA1535: 7.14E+00 (2)	79,78
Formaldehyde	2.14E-02	2	4.26E+00	66 ⁶	7.42E+00	66 ⁶	TA102: 4.10E+01 (2)	66 ⁶ , 74
							TA104: 1.28E+02	66 ⁶
							TA102: 3.50E+03	90
							TA97: 2.71E+04	90
N-(5-nitro-2-furylidene)-3-amino-2-oxazolidone (Parazolidone)	6.01E+02 (2)	3,90	4.65E+04	81				
Paryldiene derivative #2	4.43E+01 (3)	35,64,91			5.56E+00	37 ⁶		
Paryldiene derivative #3	2.74E+01 (2)	35			3.80E+00	37 ⁶		

Nitrovin	5.81E+01 (5)	3,35,91,64,65	2.23E+03	117	1.76E+03	117	TA102: 5.36E+04	82
Ofloxacin	7.78E+01	82						
N-(2-Hydroxyethyl)-3-methyl-2-quinazolin	3.01E-01	2	2.40E+02	80	3.42E+01	80		
carbamate (Olanzapine)								
Phenobarbital-1,2-diol-3,4-oxide (anti)	4.99E+00	73	1.65E+03	73	7.56E+01	73	TA104: 1.16E+03	73
							TA97: 4.83E+02	73
							TA104: 6.15E+02	73
							TA97: 6.81E+02	73
Phenanthrene-1,2-diol-3,4-oxide (syn)	1.86E+00	73	1.40E+03	73	1.04E+02	73		
Fluorist	8.79E-02	47	2.36E+00	6			TA1535: 8.08E-02	7
6-Picouic oxide	1.20E-02	7	1.57E+00	7			TA102: 7.18E+01 (2)	94,100
Potassium Sodium Dichromate(VI)ate	1.73E-02 (4)	95,44,96,97†	1.59E+01 (3)	8,98,99			TA1537: 5.84E-01	99
							TA1535: 6.30E-01	98
							TA1537: 1.19E+00	98
Potassium Chromate(VI)ate	8.36E-03 (3)	95,44,97†	1.14E+01 (3)	8,98,99	1.33E+00	98		
8-propyl octane	5.40E-01 (2)	1,32	7.02E+02 (3)	4,15,8			TA1535: 7.71E+01	4
Propyl-methylphenyl phosphine	7.98E-03	10	1.43E-01	10*				
Propyl ketamine	2.63E-02	1			1.87E+01	118		
Quercetin	1.22E-01 (3)	118,105,119	6.47E-01	80	4.69E-01	80		
Quinoxaline-1-oxide	6.54E-03	2	8.03E+01	80	1.14E+01	80		
Quinoxaline-1,4-dioxide	1.16E-01	2			3.84E-05	118		
Red wine extract (Barrada Region, Portugal)	6.27E-07	118			2.95E+01	120	TA102: 1.55E+00	120
Rodenticide	9.67E-02	55	4.26E+00	120	1.67E-02	145		
Synapse oxide	1.17E-02	7	2.65E+00 (4)	8,7,4,145	2.43E+00	121		
Urban air particulates (Paris)	5.35E-03	121	1.73E+00	121	2.19E+00	72	TA1537: 2.30E+01	72
Ventricolom A	9.23E-02	70	3.16E+00	72				

Appendix 4, Part 1 continued....

B. Substances that require metabolic activation (Aroclor 1254 induced rat S9 unless otherwise indicated).

Compound Name	SOS	Chromotest	Genotoxic Potency (IP per µg per ml.)	TA100	References	TA98	References	Ames Test Mutagenic Potency (His revertants per µg per ml.)	Other Strains	References
1,2-Dichloro-3-chloroquinone	8.12E-02	3		2.38E+01 (3)	5,6***,83	2.79E-01	83		TA97: 4.03E+00	83
1-Nitropyrene	7.91E-02 (2)	11,18		7.83E+02	18	2.59E+03	18		TA1535: 5.50E+00	83
1-Methyl-2-naphthylamine	1.49E-01	122		8.44E+01 (2)	123,124	4.08E+01	124		TA1538: 1.11E+03	18
1-Nitropyrene	7.12E-02	1		4.83E+00 (2)	4,83	1.34E+01	8		TA97: 3.25E+02	124
2,7-Dichloro-9-fluorenone	6.18E-01 (2)	11,18		2.67E+02	18	1.22E+04	18		TA1538: 2.13E+04	18
2-Acetylaminofluorene	6.69E-03 (2)	2,3		2.65E+01	128				TA1537: 4.32E+00	126
									TA1538: 1.02E+03 (2)	4,126
2-Aminonaphthalene	2.87E+00 (6)	2,44,45,47,3		2.26E+03 (9)	40,41,43*,46*,24*,48,61,34	3.19E+03 (6)	4,40,43*,8,34,125		TA97: 3.14E+02	40
									TA1535: 1.58E+02 (2)	126,34
									TA1537: 2.27E+01	126
									TA1538: 1.51E+03	126
2-Aminodibenzene	1.18E-01 (2)	1,3		8.17E+02	22	2.18E+03 (2)	22,8		TA102: 7.05E+01	22
									TA97: 3.14E+02 (2)	9,22
									TA1537: 1.66E+01 (2)	9,126
									TA1538: 2.22E+03 (3)	4,126

2-Methylanthracene	1.96E-01	127	2.70E+01	124			TA97: 1.67E+02 (2)	124,126
2-Methylphenanthrene	2.46E-01	127					TA97: 1.09E+02	127
2-Naphthylamine	3.38E-01 (2)	2,3	1.54E+02 (2)	4,128		2.18E+01	125	126
2-Nitro-5-methoxy-benzofuran (R5255)	4.04E+00	28	2.24E+03	28		5.88E+01	28	
2-Nitro-7-methoxynaphthofuran (R7000)	3.04E+01 (2)	28,29	3.93E+05	28		3.09E+04 (2)	28,29	
2-Nitro-naphthofuran (R6597)	3.40E+01	28	2.04E+04	28		2.17E+03	28	
2-Nitronaphthofuran R7419	2.78E+00	9	2.80E+03	28		2.28E+03	28	
2-Nitronaphthofuran R7489	1.19E+02	30				5.17E+04	30	
2-Nitronaphthofuran R7490	6.20E-02	30				5.36E+01	30	
2-Nitronaphthofuran R7646	3.61E+03	40				1.30E+05	29	
2-Nitronaphthofuran R7648	9.36E+01	40				4.34E+03	29	
2-Nitronaphthofuran R7691	2.51E+02	30				3.39E+05	30	
2-Nitronaphthofuran R7692	1.86E-01	30				6.69E+01	30	
2-Nitronaphthofuran R7707	2.73E+02	40				2.97E+04	29	
2-Nitronaphthalene	7.62E-02 (2)	11				1.26E+00 (2)	125	
3,2-Dimethyl-4-aminobiphenyl	1.74E-02	3	1.03E+03	4				
3-Methylcholanthrene	4.67E-01 (5)	1,2,127,21,32	3.07E+02 (5)	4,128,8,129				TA1537: 4.27E+00
								126
3-Nitrofluoranthene	2.03E-01 (2)	11,18	3.51E+03	18		5.67E+03	18	TA1538: 2.61E+01
5-Nitroquinoline	9.37E-02 (2)	11,18	3.51E+03	18		2.84E+03 (2)	11,18	18
7,12-Dimethylbenz(a)anthracene	4.51E-01 (5)	1,2,127,3,32	1.51E+02 (6)	4,128,124,8,61,129		7.56E+00	124	TA1538: 4.32E+03
								18
								TA97: 1.62E+02 (2)
								124,127
								TA1537: 1.16E+01
								126
								TA1538: 2.81E+00
								126
								TA97: 1.53E+02 (2)
								124,127
9-Methylanthracene	3.11E-01	30	4.31E+01	124		5.60E-01	124	
Acridine orange	3.55E-02	3	4.82E+02	4				
Alfatoxin B1	1.42E+01 (2)	108,70				1.21E+04 (2)	4,130	
Alfatoxin B1	1.34E+02 (10)	1,131,14,108,70,65,32,53,54	4.49E+04 (10)	4,15,128,71,5,8,61,132,72,62		2.27E+04 (6)	28,71,130,133,72,62	TA1537: 2.23E+02 (2)
								72,126
Alfatoxin B2	3.18E+00 (2)	1,70	2.98E+03 (2)	4,15		4.86E+01	130	
Alfatoxin G1	6.39E+00 (2)	1,70	3.89E+03 (2)	15,132		8.69E+02 (3)	4,71,130	
Alfatoxin M1	2.84E+01	1	8.10E+01	132		8.32E+02 (2)	4,130	
Anthranthrene	2.84E-01	127	1.87E+01	129				
Bulchinal	4.17E+01	34	9.87E-01	34		3.66E-01	34	
Benzo(a)anthracene	4.58E-02 (2)	2,127	1.28E+02 (3)	4,128,8				TA102: 6.88E+02
Benzo(a)pyrene	2.176E+00 (15)	1,2,14,44,30,127,134,18,109,51,52,122,102,32	9.90E+02 (11)	15,4,128,124,18,22,8,61,129,62		1.22E+03 (9)	124,135,30,18,22,134,125,13	22
								TA97: 1.57E+03 (3)
								124,22,9
								TA1537: 6.48E+01
								126
								TA1538: 6.64E+02 (2)
								126,18
Benzo(b)fluoranthene	1.09E-01	127	3.18E+01	136				
Benzo(b)fluorene	6.10E-02	127	2.00E+00	123				
Benzo(e)pyrene	8.66E-02 (2)	14,127	3.23E+01 (4)	4,124,8,129		3.54E+01	124	TA97: 2.49E+02
Benzo(g,h)perylene	5.40E-02	127	1.63E+01 (2)	124,129		1.88E+01	124	TA97: 1.20E+02 (2)
								124,127
								TA1537: 1.69E+01
								137
								TA1538: 1.69E+01
								137
Benzo(f)fluoranthene	5.68E-01	127	8.29E+00	136				
Captao	7.51E+00	47	4.03E+01 (2)	86,6				TA1535: 2.71E+01
Chrysene	2.25E-01 (2)	127,3	2.04E+02 (3)	4,124,137		1.29E+00	137	78
Coal Tar Extract (SRM 1597) ²⁰⁰	2.15E-02 (3)	85	4.89E+01	86		2.03E+01	86	TA97: 2.11E+02 (2)
Dibenz(a,c)anthracene	1.77E-01	127	8.98E+02 (3)	4,128,129				124,127
Dibenz(a,h)anthracene	8.27E-02 (2)	127	1.09E+02 (7)	128,129,4				137
Dibenz(a,i)pyrene	4.94E-01	127,122	1.60E+02 (3)	4,128,138		3.72E+02	138	
Diesel Particulate Matter (SRM 1650) ²⁰⁰	4.04E-03 (2)	85	1.24E+01	86		5.38E+00 (4)	86	
Dihydrovalerone	6.15E+01 (2)	2,34	2.70E+00 (2)	34,89				TA1535: 7.04E-01(4)
Dimethyl carbamoyl chloride	1.55E-02	1	2.09E+00	87		2.08E-02	87	87,38
								87,38

Fluorophore		8.64E-01 (2)	127,122	3.45E+01	124	4.40E+01	124	TA1537: 2.41E+02 (2)	124,127
<i>N</i> -(<i>tert</i> -N-methoxyflurocapro) polychlorinated (Folpet)		6.67E+00	47	2.02E+01 (3)	4,6,78			TA1535: 1.76E+01	78
1-methyl-3- <i>nitro</i> imidazole-1-ethanol		2.64E+01	34	1.48E+00	34	2.38E-01	34	TA1538: 1.08E+00	78
(<i>N</i> -nitroimidazole)		1.85E-03	105	2.15E+01	106				
<i>N</i> -Nitrooxaphthalene		7.75E-03	1						
<i>N</i> -Nitrooxipiperidine		1.58E-02	1	3.50E-01 (2)	4,87			TA1530: 1.39E+00	4
<i>N</i> -Nitrooxibutylamine		1.14E-02	1					TA1535: 1.38E-01	139
<i>N</i> -Nitrooxipropylamine		4.61E-02	1					TA1535: 5.94E-01	82,140
<i>N</i> -Nitrooxidibutylamine		1.76E-01	1					TA1537: 2.08E+00	87
<i>N</i> -Nitrooxidibutylamine		1.62E-02	1					TA1530: 2.56E+00	4
Nitrooxazole		2.31E-02	105					TA1530: 1.66E+00	4
Nitroimidazole derivative #1		7.46E-01	116						
Nitroimidazole derivative #2		4.68E-02	116						
Nitroimidazole derivative #3		1.83E-01	116						
Nitroimidazole derivative #4		9.33E-01	116						
Nitroimidazole derivative #5		6.16E-01	116						
Nitroimidazole derivative #6		1.64E-01	116						
Nitroimidazole derivative #7		7.73E-01	116						
Nitroimidazole derivative #8		3.96E-01	116						
Nitroimidazole derivative #9		1.81E+00	116						
Nitroimidazole derivative #10		5.12E+00	116						
Nitroimidazole derivative #11		7.96E-02	116						
Nitroimidazole derivative #12		4.17E-01	116						
Nitroimidazole derivative #13		1.29E+00	116						
Nitroimidazole derivative #14		6.69E-02	116						
Nitroimidazole derivative #15		5.82E-01	116						
Nitroimidazole derivative #16		2.61E+00	116						
Nitroimidazole derivative #17		1.08E+01	116						
Nitroimidazole derivative #18		1.18E+01	116						
Nitroimidazole derivative #19		1.90E+00	116						
Nitroimidazole derivative #20		1.54E-01	116						
Nitroimidazole derivative #21		2.72E+00	116						
Nitroimidazole derivative #22		1.22E-01	116						
Nitroimidazole derivative #23		1.04E+02	116						
Nitroimidazole derivative #24		1.83E+00	116						
Nitroimidazole derivative #25		5.82E+00	116						
Nitroimidazole derivative #26		2.29E+01	116						
Nitroimidazole derivative #27		5.33E+01	116						
Nitroimidazole derivative #28		7.11E+00	116						
Nitroimidazole derivative #29		1.33E-02	116						
Nitroimidazole derivative #30		2.05E-02	116						
Nitroimidazole derivative #31		5.57E-03	116						
Nitroimidazole derivative #32		2.65E-01	116						
Nitroimidazole derivative #33		4.10E-01	116						
Nitroimidazole derivative #34		1.42E+00	116						

Nitrocinclazole derivative #41	3.82E-01	116	8.85E+02	116*		TA1535: 1.83E+01	4
Nitrocinclazole derivative #44	2.71E-01	116	1.20E+03	116*		TA1535: 1.62E+02	72
Nitrocinclazole derivative #45	1.50E+00	116	6.87E+02	116*		TA97: 1.18E+02 (2)	124,127
Nitrocinclazole derivative #46	2.45E-01	116	1.17E+02	116*		TA102: 4.89E+00 (2)	94,100*
Nitrocinclazole derivative #47	5.03E-02	116	1.81E+02	116*		TA97: 1.42E+02	9
Nitrocinclazole derivative #48	2.10E-01	116	2.51E+02	116*		TA1537: 1.50E+01	9
Nitrocinclazole derivative #49	5.99E+01 (2)	91,64	4.55E+02	117	3.07E+02	TA1538: 3.51E+02 (2)	4,9
Methyl 4-(2-chloroethyl)nitrocin (Nitrogen mustard)	1.15E-01	1					
Phenanthrene	6.90E+00	141					
Potassium Sodium dichromate(VI) 100	2.18E-01	127	3.00E+00	124	3.46E+00 (2)		
Proflavine	5.67E-03 (2)	44,95	1.58E+01	98			
	6.32E-02	3					
Quercetin	4.64E-02 (4)	118,105,3,119					
5-hydroxytryptophan	1.02E+00	70	3.84E+03 (2)	4,72	4.09E+01 (2)	118,8	
(1-aminoo-1,4-dimethyl-5H-pyridin-4,3-b)iodole (TRP-P1)	3.96E+01	131	4.59E+03	62	7.46E+04 (3)	71,133,72	
(3-aminoo-1-methyl-5H-pyridin-4,3-b)iodole (TRP-P2)	3.02E+01 (2)	131,3	4.86E+03	62	2.26E+05 (3)	142,8,62	
Triphenylene	6.89E-01	129	2.91E+01	137	4.79E+00	137	
Urban Air Particulates (SRM 1649) ⁷²⁰	2.38E-04	85	2.75E-01	86	4.82E-01 (4)	86	
Urine (after coal tar treatment)	3.02E-07	44			2.13E-03	44	
Smoker's urine #1	4.46E-08	44			1.46E-05	44	
Smoker's urine #2	2.64E-08	44			5.39E-06	44	
Valerine	2.86E+01 (2)	2,34	6.18E-01 (2)	34,89			
Veratrolin A	1.04E-01	70	5.03E+01	72	1.35E+03 (2)	133,72	
					TA1537: 2.42E+01	72	

* - mixture of (E)-2-chloro-3-(dichloromethyl)-4-oxobutenoic acid + 5% (Z)-2-chloro-3-(dichloromethyl)-4-oxobutenoic acid.

** - mixture of *cis* and *trans* isomers.

*** - data for both technical grade and pure 1,2-Dibromo-3-chloropropane.

□ - mouse S9 used in activation mixture.

□ - US National Institute of Standards and Technology standard reference materials.

References: (1) Quillardet *et al.*, 1985; (2) Von Der Hude *et al.*, 1988; (3) Ohta *et al.*, 1984; (4) McCann *et al.*, 1975; (5) McCann and Ames, 1977; (6) Moriya *et al.*, 1983; (7) Von der Hude *et al.*, 1990; (8) deFlora *et al.*, 1984; (9) Levin *et al.*, 1982a; (10) Eder *et al.*, 1989; (11) Merisch-Sundermann *et al.*, 1991a; (12) Merisch-Sundermann *et al.*, 1981; (13) Pedersen and Siak, 1981; (14) Quillardet, pers. comm.; (15) Quillardet *et al.*, 1982; (16) McCoy *et al.*, 1981a; (17) El-Rayoumy *et al.*, 1981; (18) Schleibinger *et al.*, 1989; (19) De Lorenzo *et al.*, 1977; (20) McCoy *et al.*, 1981b; (21) Merisch-Sundermann *et al.*, 1991b; (22) Maron and Ames, 1983; (23) Eder *et al.*, 1990; (24) Henschler *et al.*, 1991; (25) Vasileva *et al.*, 1990; (26) Vasileva *et al.*, 1989; (27) Dunkel *et al.*, 1985; (28) Amate *et al.*, 1986; (29) Quillardet *et al.*, 1988; (30) Quillardet *et al.*, 1991; (31) Quillardet *et al.*, 1986; (32) Marzin *et al.*, 1986; (33) Karpinsky *et al.*, 1990; (34) Von der Hude *et al.*, 1986; (35) Sturak *et al.*, 1986; (36) Yabagi *et al.*, 1976; (37) Sturak *et al.*, 1988; (38) Eder *et al.*, 1992; (39) McDaniels *et al.*, 1990; (40) Tikkonen and Krenberg, 1980; (41) Deininger *et al.*, 1990; (42) Venitt and Boerwirth, 1986; (43) Eder *et al.*, 1991a; (44) Venitt *et al.*, 1991a; (45) Junz *et al.*, 1989; (46) Junz *et al.*, 1991; (47) Xu and Schurr, 1990; (48) Ilahn *et al.*, 1991; (49) Junz *et al.*, 1989; (50) Junz *et al.*, 1990; (51) Scheunwiler *et al.*, 1986; (52) Bailer *et al.*, 1987; (53) Von Poser *et al.*, 1990; (54) Moreno *et al.*, 1991; (55) Valis *et al.*, 1990; (56) daSilva *et al.*, 1994; (57) Nair *et al.*, 1991; (58) Nylund *et al.*, 1992; (59) Eder *et al.*, 1989b; (60) Venitt and Crofton-Sleigh, 1987; (61) Ames *et al.*, 1975; (62) Sugimura and Sato, 1983; (63) Benedict *et al.*, 1977; (64) Mierkus *et al.*, 1988; (65) Vojtejkova *et al.*, 1986; (66) Marnett *et al.*, 1985; (67) Kohn *et al.*, 1988; (68) Matheson *et al.*, 1978; (69) Kozrak *et al.*, 1978; (70) Krivobok *et al.*, 1987; (71) Ueno *et al.*, 1978; (72) Wehner *et al.*, 1988; (73) Ohta *et al.*, 1982b; (74) Levin *et al.*, 1982b; (75) Ruiz-Rubio *et al.*, 1985; (76) Dale *et al.*, 1989; (77) Legault *et al.*, 1994; (78) Simmon *et al.*, 1977; (79) Shirasu *et al.*, 1976; (80) Beal *et al.*, 1981; (81) Ohta *et al.*, 1980; (82) Yzem *et al.*, 1990; (83) Zeiger *et al.*, 1988; (84) Paul *et al.*, 1973; (85) White, unpublished; (86) Claxton (95) Oliver and Marzin, 1987; (96) Fish *et al.*, 1987; (97) Llagostera *et al.*, 1986; (98) Pettilli and deFlora, 1978; (99) Pettilli and deFlora, 1977; (100) Benicelli *et al.*, 1988; (101) Ohshima *et al.*, 1989; (102) Tudek *et al.*, 1988; (103) Levin and Ames, 1986; (104) Eder *et al.*, 1991b; (105) Dayan *et al.*, 1987; (106) Dayan *et al.*, 1982; (107) Widdick and Edwards, 1991; (108) Aulfray and Boudibonnes, 1987; (109) Rempola *et al.*, 1986; (110) Kalinina *et al.*, 1992; (111) Yano and Ito, 1977; (112) Dayan *et al.*, 1985; (113) Kunkalova *et al.*, 1986; (114) Goodman *et al.*, 1977; (115) Ferrera *et al.*, 1988; (116) deMeo *et al.*, 1992; (117) Jorner *et al.*, 1977; (118) Rueff *et al.*, 1986; (119) Llagostera *et al.*, 1987; (120) Valis *et al.*, 1990; (121) Courtois *et al.*, 1988; (122) Merisch-Sundermann *et al.*, 1993; (123) LaVoie *et al.*, 1980; (124) Sakai *et al.*, 1985; (125) Ito *et al.*, 1981; (126) Ames *et al.*, 1973a; (127) Merisch-Sundermann *et al.*, 1992a; (128) Baker *et al.*, 1980; (129) Anderson *et al.*, 1978; (130) Abbey *et al.*, 1982; (131) Thybuck-Lambay *et al.*, 1986; (132) Guntoro *et al.*, 1978; (133) Wong *et al.*, 1977; (134) deMeo *et al.*, 1988; (135) Ikenmura, 1981; (136) Hecht *et al.*, 1979; (137) LaVoie *et al.*, 1982; (138) Wood *et al.*, 1981; (139) Chamley and Archer, 1977; (140) Rao *et al.*, 1979; (141) Aulfray and Boudibonnes, 1988; (142) Ishii *et al.*, 1980; (143) Lecomte, 1984; (144) Mierkus *et al.*, 1986; (145) Drinkwater *et al.*, 1978; (146) Bridges and Mollerhead, 1977.

Appendix 4 Part II - Genotoxic and Carcinogenic Potency Data -

Genotoxic and carcinogenic potency data. Numbers in brackets denote sample size. Where not indicated, samples size was 1. SOS Chromotest genotoxic potency values are arithmetic mean values expressed as Induction factor units per μg of sample per assay mL. Data from the literature were standardized to an assay volume of 1.0mL. If the assay volume was not indicated, the volume recommended by Quillardet and Hofnung (1983) was assumed (ie. 0.62 mL). SOS genotoxicity was measured using *Escherichia coli* PQ37 unless otherwise indicated. Carcinogenic potency values were measured using long-term, chronic animal experiments. Carcinogenic potency values are geometric means of significant positive responses. Significant positive responses are those where the slope of the dose-response relationship was found to be significantly different from zero. Carcinogenic potency is expressed as TD50, the dose in mg per kg body weight per day that reduces the probability of remaining tumor-free at the end of an experiment (standard life-span for the animal) by 50% (ie. the Tumorigenic dose 50% described by Peto *et al.*, 1984). All carcinogenic potency data were obtained from the Carcinogenic Potency Database and its supplements (Gold *et al.*, 1984; Gold *et al.*, 1986; Gold *et al.*, 1987; Gold *et al.*, 1990; Gold *et al.*, 1993). See above for reference legend.

Compound Name	Animal	Route ^a	TD50 (mg per kg per day)	SOSIP (IF per μg per mL)	SOS References	S9 ^{b,c}
1,2-Dibromo-3-chloropropane	Mouse	Gav	9.00 (5)	0.081	3	Yes
	Mouse	Inh	5.62 (31)			
	Rat	Gav	1.98 (9)			
	Rat	Inh	1.31 (36)			
1,2-Dibromoethane	Mouse	Gav	5.78 (12)	0.0034 (3)	1,2,3	No
	Mouse	Inh	66.07 (33)			
	Mouse	Wat	36.16 (13)			
	Rat	Gav	3.98 (13)			
	Rat	Inh	12.78 (43)			
1,2,3,4-Diepoxybutane	Rat	Inh	180.96 (8)	0.066 (2)	2,7	No
1,3-Dichloropropene (Telone II)	Mouse	Gav	106.22 (10)	0.0024	2	No
	Rat	Gav	95.42 (10) ³			
1,3-Propane sultone	Rat	Gav	4.15 (3)	0.11 (2)	1,14	No
2-Amino-5-nitrothiazole	Rat	Est	42.26 (6)	0.19	2	No
2-Naphthylamine	Mouse	Est	51.58 (4)	0.34 (2)	1,3	Yes
	Mouse	Gav	20.50 (1)			
	Primate	Mix	5.74 (1)			
	Rat	Gav	61.6 (1)			
2-Acetylaminofluorene	Hamster	Est	16.64 (3)	0.0067 (2)	2,3	Yes
	Mouse	Est	54.02 (47)			
	Mouse	Gav	12.90 (1)			
	Rat	Est	3.89 (6)			
	Rat	Gav	0.56 (10)	0.47 (5)	1,2,127,21,32	Yes
3-Methylcholanthrene	Mouse	Est	0.16 (2)	0.45 (5)	1,2,127,3,32	Yes
7,12-Dimethylbenz(a)anthracene	Rat	Gav	58.66 (12)	0.0060	143	No
8-Methoxypsoralen	Rat	Wat	36.77 (2)	0.037	23	No
2-Propenal (Acrolein)	Rat	Est	0.0090 (2)	14.16 (2)	108,70	Yes
Aflatoxinol	Mouse	Est	0.027 (1)	134.39 (10)	1,131,14,108,70	Yes
Aflatoxin B ₁	Mouse	Ipj	0.0042 (2)		65,32,53,54	
	Primate	Mix	0.070 (8)			
	Rat	Est	0.0085 (14)			
Azaserine	Rat	Ipj	1.31 (3)	2.72 (2)	1,3	No
Benzo(a)pyrene	Mouse	Est	1.86 (1)	2.17 (15)	1,2,14,44,30,127,102,32	Yes
	Mouse	Wat	11.68 (2)		18,109,51,52,135,122	
	Mouse	Orl	34.00 (1)	0.16	47	No
Hydroxydimethylamine oxide (Cacodylic acid)	Rat	Inh	0.048	0.98	14	No
Cadmium chloride	Mouse	Est	664.79 (13)	10.47	3	No
Captan	Mouse	Est	6223.40 (3)	14.08 (3)	47,3,77	No
	Mouse	Orl	60.49 (2)			
	Rat	Est	253.00 (1)			

Chromium acetate	Rat	Wat	1.61 (1)	0.0019	44	No
Dacarbazine	Mouse	Ipj	3.36 (11)	2.99 (2)	67	No
	Rat	Est	2.70 (3)			
Dibenz(a,h)anthracene	Mouse	Wat	5.88 (1)	0.083 (2)	127	Yes
Dimethyl carbamoyl chloride	Mouse	Ipj	4.96 (2)	0.011	1	No
Epichlorohydrin	RAT	Gav	2.55 (1)	0.030 (5)	2,14,48,21,7	No
Ferrie dimethyldithiocarbamate (Ferbam)	Mouse	Orl	3.96 (2)	0.57	47	No
N-(trichloromethylmercapto)-phthalimide (Folpet)	Mouse	Orl	116.97 (2)	14.03	47	No
2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (Furylfuramide)	Hamster	Est	99.23 (4)	1119.40 (2)	35,3	No
	Mouse	Est	200.00 (11)			
	Rat	Est	249.41 (8)			
Hydrazine	Hamster	Inh	15.65 (2)	0.34	14	Yes
	Mouse	Inh	7.78 (1)			
	Mouse	Wat	4.57 (6)			
	Rat	Inh	3.82 (10)			
Methyl-methanesulfonate	Mouse	Wat	76.70 (2)	0.19 (11)	1,2,14,43,10,101,46,21,102,24	No
2-Methyl-5-nitroimidazole-1-ethanol (Metronidazole)	Mouse	Est	750.48 (3)	0.0036	105	No
	Rat	Est	595.59 (12)			
Mitomycin C	Rat	Ipj	0.0017 (7)	70.31 (7)	1,14,44,108,3,109,65	No
N-Butyl-N'-nitrosourea	Rat	Wat	6.69 (27)	0.026	3	No
N-Methyl-N'-nitro-N-nitrosoguanidine	Rat	Wat	1.68 (26)	3.41 (9)	1,2,82,14,44,3,102,65,144	No
N-Nitrosodipropylamine	Rat	Gav	0.26 (3)	0.046	1	Yes
N-Nitrosomorpholine	Hamster	Wat	5.61 (6)	0.0078	1	Yes
	Rat	Wat	0.71 (16)			
N-Nitrosopiperidine	Hamster	Wat	78.09 (8)	0.016	1	Yes
	Mouse	Est	2.77 (4)			
	Primate	Est	19.19 (2)			
	Primate	Ipj	0.67 (2)			
	Rat	Wat	1.52 (3)			
N-Ethyl-N'-nitro-nitrosoguanidine	Mouse	Wat	4.60 (5)	2.74 (2)	2,3	No
N-Ethyl-N'-nitrosourea	Rat	Wat	1.14 (15)	0.039 (2)	2,3	No
N-Methyl-N'-nitrosourea	Primate	Est	4.42 (5)	0.015 (2)	2,3	No
N-Nitrosodibutylamine	Mouse	Est	3.04 (6)	0.011	1	Yes
	Rat	Gav	1.02 (4)			
N-Nitrosodiethylamine	Primate	Ipj	0.031 (5)	0.18	1	Yes
	Rat	Wat	0.040 (16)			
N-Nitrosodimethylamine	Mouse	Gav	0.17 (7)	0.016	1	Yes
	Mouse	Wat	0.32 (1)			
	Rat	Est	0.67 (8)			
	Rat	Gav	0.27 (8)			
	Rat	Wat	0.15 (3)			
Nalidixic acid	Mouse	Est	1177.80 (3)	0.35	82	No
	Rat	Est	236.68 (4)			
1-(5-nitro-2-furfurylideneamino)-hydantoin (Nitrofurantoin)	Mouse	Est	1794.4 (4)	37.66 (3)	35,30,113	No
	Rat	Est	181.12 (4)			
5-Nitro-2-furaldehyde semicarbazone (Nitrofurazone)	Mouse	Est	52.34 (5)	38.67 (2)	30,90	No
	Rat	Est	34.32 (7)			
2-Chloro-N-(2-chloroethyl)-N-methylethanamine (Nitrogen mustard)	Rat	Ipj	0.021 (3)	1.02 (2)	1,14	No
Proflavine	Mouse	Est	26.40 (2)	0.063	3	Yes
	Rat	Est	239.52 (3)			
8-propiolactone	Mouse	Gav	1.34 (4)	0.54 (2)	1,32	No
	Rat	Gav	1.47 (2)			
Quercetin	Mouse	Est	4240.00 (1)	0.12 (3)	118,105,119	No
	Rat	Est	11.83 (3)			
5-(2-Propenyl)-1,3-benzodioxole (Safrole)	Mouse	Est	121.95 (9)	1.7E-05	14	No
	Mouse	Gav	29.85 (2)			
	Mouse	Orl	50.96 (5)			

Sterigmatocystin	Rat	Eat	548.76 (8)	1.02	70	Yes
	Mouse	Eat	2.40 (6)			
	Rat	Eat	0.26 (5)			
	Rat	Gav	0.32 (1)			
Styrene oxide	Mouse	Gav	260.72 (7)	0.012	7	No
	Rat	Gav	146.64 (30)			
(3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole) (TRP-P1)	Mouse	Eat	43.60 (3)	39.61	131	Yes
	Rat	Eat	0.58 (2)			
(3-amino-1-methyl-5H-pyrido[4,3-b]indole) (TRP-P2)	Mouse	Eat	23.66 (2)	30.25 (2)	131,3	Yes
	Rat	Eat	5.32 (2)			
Stannous chloride	Mouse	Eat	1505.30 (4)	0.0054	95	No
	Rat	Eat	322.89 (3)			

* Routes of Administration (Gold *et al.*, 1984).

Eat = diet

Gav = gavage

Inh = inhalation

Ipj = Intraperitoneal injection

Ivj = Intravenous injection

Mix = Multiple routes

Orl = Gavage preweanling, followed by diet

Wat = Water

** Indicates whether or not genotoxicity requires S9 metabolic activation.

APPENDIX 5

Actual names of industries examined in chapters 3 and 4.

Name Used	Actual Name
<u>Aluminum Refineries--</u>	
Aluminum #1	Aluminerie de Bécancour, Bécancour
Aluminum #2- Sample 1	Alcan, Usine Beauharnois
Aluminum #2- Sample 2	Alcan, Usine Beauharnois
Aluminum #3	Alcan, Alma
Aluminum #4	Alcan, La Baie
Aluminum #5	Reynolds Cap-de-la-Madeleine
Aluminum #6- Sample 1	Reynolds, Baie Comeau
Aluminum #6- Sample 2	Reynolds, Baie Comeau
<u>Inorganic Chemical Production Facilities--</u>	
Inorganic #1	Albright & Wilson, Varennes
Inorganic #2	ICI Canada, Bécancour
Inorganic #3- Sample 1	Kronos Canada, Varennes pt. 1
Inorganic #3- Sample 2	Kronos Canada, Varennes pt. 4
Inorganic #4	PPG Canada, Beauharnois
Inorganic #5- Sample 1	Tioxide Canada, Tracy- jour 1
Inorganic #5- Sample 2	Tioxide Canada, Tracy- jour 3
<u>Non-Aluminum Metal Refineries--</u>	
Metal #1	Acier Atlas, Tracy
Metal #2	Canadian Copper Refineries, Montréal East
Metal #3- Sample 1	Zinc Électrolytique du Canada, Valleyfield pt. 3
Metal #3- Sample 2	Zinc Électrolytique du Canada, Valleyfield pt. 8
Metal #4	Niobec, St. Honoré
Metal #5	QIT- Fer et Titane, Sorel
Metal #6	Sidbec-Dosco, Contrecoeur
<u>Organic Chemical Production Facilities--</u>	
Organic #1	Alcools de Commerce, Varennes
Organic #2	Hoescht Canada, Varennes
Organic #3	Industries de Préservation du Bois, Tracy
Organic #4	Monsanto Inc, Lasalle
Organic #5	Produits Nacan, Boucherville
Organic #6- Sample 1	Union Carbide, Montréal East

Organic #6- Sample 2	Union Carbide, Montréal East
Organic #7	Prod. Chimiques Expro, Saint-Timothée

Pulp and Paper Production--

Paper #1	Abitibi-Price, Jonquière
Paper #2	Domtar Inc, Donnacona
Paper #3	Northwood Paper, Prince George, B.C.
Paper #4	Perkins Paper, Candiac
Paper #5	Rolland Paper, St. Jerome
Paper #6	Stone Consolidated, Trois Rivières

Petroleum Refining Facilities--

Petroleum #1- Sample 1	Péto-Canada, Pointe-aux-Trembles
Petroleum #1- Sample 2	Péto-Canada, Pointe-aux-Trembles
Petroleum #2	Pétromont Inc, Varennes
Petroleum #3	Shell Canada, Montréal East
Petroleum #4- Sample 1	Ultramar, St. Romuald
Petroleum #4- Sample 2	Ultramar, St. Romuald

Municipal Wastewater Treatment Plants-

Sewage #1	Communauté Urbaine de Montréal
Sewage #2	Annacis Island, British Columbia

Surface Treatment Facilities

Surface #1	Héroux, Longueuil
Surface #2	Lockweld, Candiac
Surface #3- Sample 1	Pratt & Whitney Usine 1, Longueuil pt. 1
Surface #3- Sample 2	Pratt & Whitney Usine 1, Longueuil pt. 2
Surface #4	Pratt & Whitney Usine 2, Longueuil
Surface #5	Pratt & Whitney Usine 5, St. Hubert

APPENDIX 6

Data Used to Generate Models and Figures in Chapters 3 and 4

A) Figure 1 Data--

Industry	Actual Name	Sample	S9 Activ.	Conc. (mg sample Equiv per well)†	Mean SOS Induction Factor	Std. Error
Alum Ind #2	Alcan, Alma	Aq-Acid	N	2587.50	1.2503	0.0737
Alum Ind #2	Alcan, Alma	Aq-Acid	N	5175.00	1.3323	0.1132
Alum Ind #2	Alcan, Alma	Aq-Acid	N	10350.00	1.4619	0.0165
Alum Ind #2	Alcan, Alma	Aq-Acid	N	20700.00	1.6547	0.1821
Alum Ind #2	Alcan, Alma	Aq-Acid	N	41400.00	2.1939	0.1966
Alum Ind #2	Alcan, Alma	Aq-Acid	N	82800.00	2.7267	0.0183
Alum Ind #2	Alcan, Beauham	Solids	N	0.07	1.2072	0.0070
Alum Ind #2	Alcan, Beauham	Solids	N	0.15	1.3967	0.1014
Alum Ind #2	Alcan, Beauham	Solids	N	0.30	1.6180	0.1030
Alum Ind #2	Alcan, Beauham	Solids	N	0.59	1.8982	0.1114
Alum Ind #2	Alcan, Beauham	Solids	N	1.18	1.8933	0.0510
Alum Ind #2	Alcan, Beauham	Solids	N	2.36	2.5999	0.0378
Sewage #2	CUM Sewage	Solids	N	0.21	0.8836	0.0214
Sewage #2	CUM Sewage	Solids	N	0.42	0.9205	0.0614
Sewage #2	CUM Sewage	Solids	N	0.85	1.0241	0.0354
Sewage #2	CUM Sewage	Solids	N	1.69	1.1538	0.0294
Sewage #2	CUM Sewage	Solids	N	3.38	1.5909	0.0526
Sewage #2	CUM Sewage	Solids	N	6.76	1.6622	0.2309
Org. Chem #7	Expro	Solids	Y	0.03	0.8737	0.0428
Org. Chem #7	Expro	Solids	Y	0.05	1.0969	0.0579
Org. Chem #7	Expro	Solids	Y	0.10	1.3137	0.1417
Org. Chem #7	Expro	Solids	Y	0.20	1.9269	0.0146
Org. Chem #7	Expro	Solids	Y	0.40	2.3961	0.1590
Org. Chem #7	Expro	Solids	Y	0.81	2.6009	0.1791
Petro #1	PetroCanada	Aq-Acid	N	500.00	1.1335	0.0467
Petro #1	PetroCanada	Aq-Acid	N	1000.00	1.4129	0.0170
Petro #1	PetroCanada	Aq-Acid	N	2000.00	1.8148	0.0311
Petro #1	PetroCanada	Aq-Acid	N	4000.00	2.7815	0.0164

Petro #1	PetroCanada	Aq-Acid	N	8000.00	5.1379	0.0807
Petro #1	PetroCanada	Aq-Acid	N	16000.00	8.7903	0.9307
Petro #2	Petromont	Aq-Alkaline	Y	2500.00	1.2031	0.0080
Petro #2	Petromont	Aq-Alkaline	Y	5000.00	1.2458	0.0023
Petro #2	Petromont	Aq-Alkaline	Y	10000.00	1.3157	0.0051
Petro #2	Petromont	Aq-Alkaline	Y	20000.00	1.4563	0.0605
Petro #2	Petromont	Aq-Alkaline	Y	40000.00	1.7141	0.0688
Petro #2	Petromont	Aq-Alkaline	Y	80000.00	2.5098	0.2994
P&P #6	Stone, 3-Riv	Aq-Acid	N	450.00	1.5242	0.0750
P&P #6	Stone, 3-Riv	Aq-Acid	N	900.00	1.9646	0.0959
P&P #6	Stone, 3-Riv	Aq-Acid	N	1800.00	2.3394	0.0213
P&P #6	Stone, 3-Riv	Aq-Acid	N	3600.00	4.0384	0.0183
P&P #6	Stone, 3-Riv	Aq-Acid	N	7200.00	5.1361	0.0152
P&P #6	Stone, 3-Riv	Aq-Acid	N	14400.00	5.6058	0.1637
Surface #3	Pratt & Whit pt 2	Solids	Y	0.22	1.1142	0.0016
Surface #3	Pratt & Whit pt 2	Solids	Y	0.43	1.3414	0.0211
Surface #3	Pratt & Whit pt 2	Solids	Y	0.86	1.9149	0.0359
Surface #3	Pratt & Whit pt 2	Solids	Y	1.73	2.0935	0.1046
Surface #3	Pratt & Whit pt 2	Solids	Y	3.46	2.05238	0.15784

†Concentration in mg per assay mL = conc. in mg per well x 0.2

B) Industrial effluent genotoxicity results used to generate Figures 3 through 6 and models 1 through 4 in Chapter 3 as well as Figures 1 through 3 and models 1 and 2 in Chapter 4.

Variables:

Sample-- ACID = Aqueous, acidic extract, BASE = Aqueous, alkaline extract, SOLID = Suspended solid extract.

S9 Activ. = S9 metabolic activation status. Y = +S9, N = No S9.

MaxIF = Maximum level of SOS Induction observed.

MaxTox = Maximum toxicity observed expressed as %'age of alkaline phosphatase level of solvent (DMSO) control.

Fluor. = Sample fluorescence expressed as ppb Benzo(a)pyrene fluorescent equivalents.

SRIP = SOS Response Inducing potency - expressed as IF per mg sample equivalent per assay mL for all samples (mL = g).

Ind. Type = Industry type. ALUM = Aluminum manufacture, PAPER = Pulp & Paper, INORG = Inorganic chemical manufacture, ORGCHEM = Organic chemical manufacture, SEWAGE = Municipal wastewater treatment, METAL = Metal refining and founding, SURFACE = Metal surface plating and treatment.

LOGC = Lowest observable genotoxic concentration in mg sample equivalent per assay mL.

Res. Conc = Concentration of extractable residue in mg per L for aqueous samples and mg per g dry wt. for solid samples.

Q = Mean daily discharge in m³ per day.

TSS = Total suspended solids in dry mg per L.

Industry	Sub-Samp	Sample Type	S9 Activ	MaxIF	Max Tox	Fluor	SRIP	Ind. Type	LOGC	Res. Conc	Q	TSS
Aluminum #1		ACID	N	1.39	80.0	4.80E-02	6.40E-07	ALUM	.	3.45E+00	1152	10.7
Aluminum #1		BASE	N	1.42	96.0	3.92E-02	.	ALUM	.	3.33E+00	1152	10.7
Aluminum #1		SOLID	N	1.83	65.0	2.19E+04	3.95E-01	ALUM	.	4.36E+02	1152	10.7
Aluminum #1		ACID	Y	1.09	90.0	4.80E-02	3.66E-07	ALUM	4.13E+05	3.45E+00	1152	10.7
Aluminum #1		BASE	Y	1.02	98.0	3.92E-02	3.66E-07	ALUM	4.13E+05	3.33E+00	1152	10.7
Aluminum #1		SOLID	Y	1.09	84.0	2.19E+04	1.25E-02	ALUM	5.39E+00	4.36E+02	1152	10.7
Paper #1		ACID	N	1.17	96.0	1.64E-01	1.87E-06	PAPER	4.04E+04	2.16E+00	132940	31.0
Paper #1		BASE	N	1.32	72.0	6.70E-02	5.07E-05	PAPER	2.96E+03	6.47E+00	132940	31.0
Paper #1		SOLID	N	1.35	21.0	3.98E+03	.	PAPER	.	2.43E+02	132940	31.0
Paper #1		ACID	Y	1.06	87.0	1.64E-01	3.66E-07	PAPER	4.04E+05	2.16E+00	132940	31.0
Paper #1		BASE	Y	1.09	71.0	6.70E-02	3.66E-07	PAPER	4.04E+05	6.47E+00	132940	31.0
Paper #1		SOLID	Y	1.04	44.0	3.98E+03	5.20E-04	PAPER	2.50E+01	2.43E+02	132940	31.0
Inorganic #1		ACID	N	1.73	62.0	3.70E-01	2.25E-05	INORG	2.08E+03	2.38E+00	6744	14.4
Inorganic #1		BASE	N	1.17	79.0	3.00E-01	4.21E-06	INORG	2.93E+04	2.96E+00	6744	14.4
Inorganic #1		SOLID	N	1.98	81.0	9.17E+03	2.38E-01	INORG	1.36E-01	3.80E+01	6744	14.4
Inorganic #1		ACID	Y	1.43	53.0	3.70E-01	1.06E-06	INORG	5.31E+04	2.38E+00	6744	14.4
Inorganic #1		BASE	Y	1.09	79.0	3.00E-01	3.66E-07	INORG	3.71E+05	2.96E+00	6744	14.4
Inorganic #1		SOLID	Y	1.28	88.0	9.17E+03	8.57E-02	INORG	7.25E-02	3.80E+01	6744	14.4
Aluminum #2	Sample 1	ACID	N	1.42	92.0	1.02E-01	.	ALUM	.	2.86E+00	5638	15.0
Aluminum #2	Sample 1	BASE	N	1.19	78.0	6.50E-02	4.90E-05	ALUM	2.94E+03	1.52E+00	5638	15.0
Aluminum #2	Sample 1	SOLID	N	2.59	60.0	9.65E+05	1.29E-01	ALUM	.	7.60E+01	5638	15.0
Aluminum #2	Sample 1	ACID	Y	1.06	90.0	1.02E-01	3.66E-07	ALUM	3.94E+05	2.86E+00	5638	15.0
Aluminum #2	Sample 1	BASE	Y	1.04	87.0	6.50E-02	3.66E-07	ALUM	3.94E+05	1.52E+00	5638	15.0
Aluminum #2	Sample 1	SOLID	Y	1.31	74.0	9.65E+05	5.04E-02	ALUM	.	7.60E+01	5638	15.0
Aluminum #3		ACID	N	2.73	100.0	8.80E-02	3.46E-06	ALUM	.	1.00E+01	13224	1.0
Aluminum #3		BASE	N	1.17	84.0	3.32E-01	.	ALUM	.	2.23E+00	13224	1.0
Aluminum #3		SOLID	N	1.66	62.0	1.15E+06	3.59E+00	ALUM	4.51E-02	7.11E+03	13224	1.0
Aluminum #3		ACID	Y	1.24	84.0	8.80E-02	7.60E-07	ALUM	1.65E+05	1.00E+01	13224	1.0
Aluminum #3		BASE	Y	1.03	91.0	3.32E-01	3.66E-07	ALUM	4.15E+05	2.23E+00	13224	1.0
Aluminum #3		SOLID	Y	1.33	73.0	1.15E+06	2.08E+00	ALUM	4.35E-02	7.11E+03	13224	1.0
Aluminum #4		ACID	N	1.74	86.0	7.10E-02	3.90E-06	ALUM	.	1.65E+00	250	5.0
Aluminum #4		BASE	N	1.48	88.0	1.80E-02	1.16E-05	ALUM	2.27E+04	2.02E+00	250	5.0
Aluminum #4		SOLID	N	1.87	68.0	1.58E+04	1.99E+00	ALUM	1.14E-01	3.00E+02	250	5.0

Aluminum #4		ACID	Y	1.07	85.0	7.10E-02	3.66E-07	ALUM	4.08E+05	1.65E+00	250	5.0
Aluminum #4		BASE	Y	1.12	97.0	1.80E-02	3.66E-07	ALUM	1.67E+05	2.02E+00	250	5.0
Aluminum #4		SOLID	Y	1.57	99.0	1.58E+04	1.45E-01	ALUM	7.35E-01	3.00E+02	250	5.0
Organic #1		ACID	N	1.18	84.0	9.97E-02	4.26E-07	ORGCHEM	3.96E+05	3.34E+00	900	15.0
Organic #1		BASE	N	1.35	96.0	2.30E-02	3.55E-05	ORGCHEM	1.05E+04	4.67E+00	900	15.0
Organic #1		SOLID	N	1.64	78.0	9.94E+03	1.97E+00	ORGCHEM	3.74E-01	2.04E+02	900	15.0
Organic #1		ACID	Y	1.12	85.0	9.97E-02	.	ORGCHEM	.	3.34E+00	900	15.0
Organic #1		BASE	Y	1.13	93.0	2.30E-02	3.39E-05	ORGCHEM	1.49E+03	4.67E+00	900	15.0
Organic #1		SOLID	Y	1.39	80.0	9.94E+03	3.20E-02	ORGCHEM	9.70E-01	2.04E+02	900	15.0
Sewage #2		ACID	N	1.21	12.4	1.32E+00	4.26E-07	SEWAGE	4.03E+05	2.18E+01	415870	61.5
Sewage #2		BASE	N	1.28	17.1	6.80E-01	4.26E-07	SEWAGE	4.03E+05	1.26E+01	415870	61.5
Sewage #2		SOLID	N	1.13	5.5	6.00E+03	1.58E-03	SEWAGE	4.96E+01	2.33E+02	415870	61.5
Sewage #2		ACID	Y	1.05	17.6	1.32E+00	3.66E-07	SEWAGE	4.03E+05	2.18E+01	415870	61.5
Sewage #2		BASE	Y	1.04	36.0	6.80E-01	3.66E-07	SEWAGE	4.03E+05	1.26E+01	415870	61.5
Sewage #2		SOLID	Y	1.08	21.5	6.00E+03	5.20E-04	SEWAGE	4.96E+01	2.33E+02	415870	61.5
Metal #1		ACID	N	1.29	17.3	3.43E-02	6.93E-05	METAL	1.85E+03	2.40E+00	78000	58.0
Metal #1		BASE	N	1.20	70.0	2.18E-02	.	METAL	.	6.90E+00	78000	58.0
Metal #1		SOLID	N	1.92	79.0	4.56E+04	1.72E-02	METAL	2.54E+01	7.50E+01	78000	58.0
Metal #1		ACID	Y	1.06	79.0	3.43E-02	3.66E-07	METAL	5.90E+05	2.40E+00	78000	58.0
Metal #1		BASE	Y	1.17	92.0	2.18E-02	.	METAL	.	6.90E+00	78000	58.0
Metal #1		SOLID	Y	1.36	88.0	4.56E+04	3.80E-03	METAL	6.86E+01	7.50E+01	78000	58.0
Metal #2		ACID	N	1.06	7.9	2.00E-01	4.26E-07	METAL	3.94E+05	1.70E+01	20500	2.6
Metal #2		BASE	N	1.57	100.0	9.00E-02	3.84E-05	METAL	.	1.70E+01	20500	2.6
Metal #2		SOLID	N	2.22	84.0	1.25E+04	3.32E-01	METAL	.	1.46E+03	20500	2.6
Metal #2		ACID	Y	1.11	27.0	2.00E-01	3.66E-07	METAL	3.94E+05	1.70E+01	20500	2.6
Metal #2		BASE	Y	1.14	96.0	9.00E-02	3.66E-07	METAL	3.94E+05	1.70E+01	20500	2.6
Metal #2		SOLID	Y	1.92	91.0	1.25E+04	4.16E-01	METAL	1.84E-01	1.46E+03	20500	2.6
Metal #3	Sample 1	ACID	N	1.32	86.0	2.30E-02	3.17E-05	METAL	5.97E+03	3.38E+00	160060	4.6
Metal #3	Sample 1	SOLID	N	1.33	62.0	1.96E+04	.	METAL	.	2.89E+04	160060	4.6
Metal #3	Sample 1	ACID	Y	1.07	90.0	2.30E-02	.	METAL	.	3.38E+00	160060	4.6
Metal #3	Sample 1	SOLID	Y	1.14	51.0	1.96E+04	3.30E-02	METAL	1.16E+00	2.89E+04	160060	4.6
Metal #3	Sample 2	ACID	N	1.15	89.0	7.20E-02	.	METAL	.	1.98E+00	3940	15.7
Metal #3	Sample 2	BASE	N	1.10	98.0	6.60E-02	4.26E-07	METAL	4.55E+05	1.70E+00	3940	15.7
Metal #3	Sample 2	SOLID	N	1.54	26.0	4.27E+03	4.64E-01	METAL	1.44E-01	1.05E+03	3940	15.7
Metal #3	Sample 2	ACID	Y	1.08	95.0	7.20E-02	3.66E-07	METAL	4.55E+05	1.98E+00	3940	15.7
Metal #3	Sample 2	BASE	Y	1.03	90.0	6.60E-02	3.66E-07	METAL	4.55E+05	1.70E+00	3940	15.7
Metal #3	Sample 2	SOLID	Y	1.75	22.0	4.27E+03	1.18E+00	METAL	3.19E-02	1.05E+03	3940	15.7
Sewage #1		ACID	N	1.78	41.0	8.13E-01	.	SEWAGE	.	1.92E+00	1615700	19.0
Sewage #1		BASE	N	1.42	58.0	1.18E+00	4.72E-05	SEWAGE	4.01E+03	2.16E+00	1615700	19.0

Sewage #1	SOLID	N	1.66	48.0	1.15E+04	2.86E-02	SEWAGE	5.48E+00	1.56E+02	1615700	19.0
Sewage #1	ACID	Y	1.31	69.0	8.13E-01	7.75E-07	SEWAGE	1.02E+05	1.92E+00	1615700	19.0
Sewage #1	BASE	Y	1.06	66.0	1.18E+00	.	SEWAGE	.	2.16E+00	1615700	19.0
Sewage #1	SOLID	Y	1.26	70.0	1.15E+04	1.25E-02	SEWAGE	8.45E+00	1.56E+02	1615700	19.0
Paper #2	ACID	N	1.28	69.0	1.00E+00	1.48E-06	PAPER	4.16E+02	1.42E+01	38500	92.3
Paper #2	BASE	N	1.24	70.0	5.40E-01	9.20E-07	PAPER	1.20E+05	1.42E+01	38500	92.3
Paper #2	SOLID	N	1.16	67.0	1.70E+03	1.58E-03	PAPER	6.50E+01	1.98E+02	38500	92.3
Paper #2	ACID	Y	3.99	36.0	1.00E+00	1.12E-05	PAPER	4.16E+04	1.42E+01	38500	92.3
Paper #2	BASE	Y	1.34	58.0	5.40E-01	1.54E-06	PAPER	1.63E+05	1.42E+01	38500	92.3
Paper #2	SOLID	Y	1.06	88.0	1.70E+03	4.27E-02	PAPER	6.15E+00	1.98E+02	38500	92.3
Organic #7	ACID	N	1.26	10.3	1.95E-02	9.52E-06	ORGCHEM	2.18E+04	4.10E+00	20280	2.6
Organic #7	BASE	N	1.60	11.0	1.16E-02	1.20E-05	ORGCHEM	2.84E+04	4.52E+00	20280	2.6
Organic #7	SOLID	N	2.60	61.0	4.36E+03	9.23E+00	ORGCHEM	2.09E-01	3.14E+02	20280	2.6
Organic #7	ACID	Y	1.07	20.0	1.95E-02	.	ORGCHEM	.	4.10E+00	20280	2.6
Organic #7	BASE	Y	1.21	18.0	1.16E-02	1.58E-06	ORGCHEM	3.86E+04	4.52E+00	20280	2.6
Organic #7	SOLID	Y	1.31	96.0	4.36E+03	8.93E-02	ORGCHEM	7.64E-01	3.14E+02	20280	2.6
Surface #1	ACID	N	1.46	30.0	1.63E-01	2.00E-06	SURFACE	.	8.80E+00	450	9.3
Surface #1	BASE	N	1.19	21.8	1.20E-01	.	SURFACE	.	5.76E+00	450	9.3
Surface #1	SOLID	N	4.28	53.2	5.62E+04	3.82E-01	SURFACE	.	7.32E+02	450	9.3
Surface #1	ACID	Y	1.03	52.0	1.63E-01	3.66E-07	SURFACE	3.85E+05	8.80E+00	450	9.3
Surface #1	BASE	Y	1.14	57.4	1.20E-01	6.91E-07	SURFACE	1.20E+05	5.76E+00	450	9.3
Surface #1	SOLID	Y	1.33	54.0	5.62E+04	1.15E-01	SURFACE	.	7.32E+02	450	9.3
Organic #2	ACID	N	1.36	66.0	3.75E-02	4.46E-05	ORGCHEM	3.77E+03	2.90E+00	2470	12.0
Organic #2	BASE	N	1.32	81.0	3.15E-02	4.94E-05	ORGCHEM	3.55E+03	3.98E+00	2470	12.0
Organic #2	SOLID	N	2.43	90.0	2.85E+03	8.71E-01	ORGCHEM	1.80E-02	5.30E+00	2470	12.0
Organic #2	ACID	Y	1.16	71.0	3.75E-02	3.66E-07	ORGCHEM	4.00E+05	2.90E+00	2470	12.0
Organic #2	BASE	Y	1.08	87.0	3.15E-02	3.66E-07	ORGCHEM	4.00E+05	3.98E+00	2470	12.0
Organic #2	SOLID	Y	1.32	93.0	2.85E+03	3.62E-02	ORGCHEM	2.05E+00	5.30E+00	2470	12.0
Inorganic #2	ACID	N	1.30	79.0	3.60E-02	3.20E-05	INORG	7.50E+03	2.22E+00	6600	12.0
Inorganic #2	BASE	N	1.14	74.0	1.40E-02	4.26E-07	INORG	4.16E+05	2.10E+00	6600	12.0
Inorganic #2	SOLID	N	1.49	67.0	6.58E+03	3.08E-01	INORG	2.41E-01	7.60E+02	6600	12.0
Inorganic #2	ACID	Y	1.10	87.0	3.60E-02	3.66E-07	INORG	4.16E+05	2.22E+00	6600	12.0
Inorganic #2	BASE	Y	1.06	89.0	1.40E-02	3.66E-07	INORG	4.16E+05	2.10E+00	6600	12.0
Inorganic #2	SOLID	Y	1.40	85.0	6.58E+03	7.28E-02	INORG	1.25E-02	7.60E+02	6600	12.0
Organic #3	ACID	N	1.22	88.0	1.43E-02	6.00E-06	ORGCHEM	2.12E+04	2.13E+00	22	2.0
Organic #3	BASE	N	1.45	82.0	1.23E-02	4.26E-07	ORGCHEM	4.00E+05	6.06E+00	22	2.0
Organic #3	SOLID	N	1.45	69.0	5.11E+04	3.38E+00	ORGCHEM	4.42E-02	6.77E+02	22	2.0
Organic #3	ACID	Y	1.02	97.0	1.43E-02	3.66E-07	ORGCHEM	4.00E+05	2.13E+00	22	2.0
Organic #3	BASE	Y	1.04	90.0	1.23E-02	3.66E-07	ORGCHEM	4.00E+05	6.06E+00	22	2.0

Organic #3		SOLID	Y	1.30	89.0	5.11E+04	1.46E-01	ORGCHEM	5.45E-01	6.77E+02	22	2.0
Inorganic #3	Sample 1	ACID	N	1.32	78.0	4.53E-02	.	INORG	.	3.80E+00	.	69.0
Inorganic #3	Sample 1	BASE	N	1.20	88.0	2.10E-02	8.68E-06	INORG	1.98E+04	2.30E+00	.	69.0
Inorganic #3	Sample 1	SOLID	N	2.42	76.0	1.91E+03	2.58E-01	INORG	2.00E+00	1.15E+01	.	69.0
Inorganic #3	Sample 1	ACID	Y	1.04	91.0	4.53E-02	3.66E-07	INORG	4.17E+05	3.80E+00	.	69.0
Inorganic #3	Sample 1	BASE	Y	1.09	89.0	2.10E-02	.	INORG	.	2.30E+00	.	69.0
Inorganic #3	Sample 1	SOLID	Y	1.31	90.0	1.91E+03	6.54E-03	INORG	2.15E+00	1.15E+01	.	69.0
Inorganic #3	Sample 2	ACID	N	1.08	86.0	3.70E-02	6.95E-05	INORG	1.51E+03	2.35E+00	.	73.5
Inorganic #3	Sample 2	BASE	N	1.14	90.0	1.20E-02	4.26E-07	INORG	4.05E+05	1.86E+00	.	73.5
Inorganic #3	Sample 2	SOLID	N	1.84	76.0	9.29E+02	4.67E-02	INORG	1.21E+00	1.11E+01	.	73.5
Inorganic #3	Sample 2	ACID	Y	1.09	96.0	3.70E-02	3.66E-07	INORG	4.05E+05	2.35E+00	.	73.5
Inorganic #3	Sample 2	BASE	Y	1.03	93.0	1.20E-02	3.66E-07	INORG	4.05E+05	1.86E+00	.	73.5
Inorganic #3	Sample 2	SOLID	Y	1.09	88.0	9.29E+02	.	INORG	.	1.11E+01	.	73.5
Surface #2		ACID	N	1.29	75.0	3.40E-02	.	SURFACE	.	9.30E-01	68	295.0
Surface #2		BASE	N	1.84	58.0	9.30E-02	.	SURFACE	.	9.60E-01	68	295.0
Surface #2		SOLID	N	2.23	65.0	1.67E+03	1.51E-02	SURFACE	.	5.17E+00	68	295.0
Surface #2		ACID	Y	1.06	88.0	3.40E-02	3.66E-07	SURFACE	8.85E+05	9.30E-01	68	295.0
Surface #2		BASE	Y	1.08	67.0	9.30E-02	3.66E-07	SURFACE	8.85E+05	9.60E-01	68	295.0
Surface #2		SOLID	Y	1.27	100.0	1.67E+03	6.69E-04	SURFACE	3.05E+01	5.17E+00	68	295.0
Organic #4		ACID	N	4.80	52.0	8.90E-02	.	ORGCHEM	.	4.20E+02	.	246.0
Organic #4		BASE	N	2.92	43.0	4.20E-02	.	ORGCHEM	.	1.47E+02	.	246.0
Organic #4		SOLID	N	.	83.0	2.87E+03	.	ORGCHEM	.	1.11E+03	.	246.0
Organic #4		ACID	Y	5.40	52.0	8.90E-02	.	ORGCHEM	.	4.20E+02	.	246.0
Organic #4		BASE	Y	1.00	41.0	4.20E-02	.	ORGCHEM	.	1.47E+02	.	246.0
Organic #4		SOLID	Y	.	37.0	2.87E+03	.	ORGCHEM	.	1.11E+03	.	246.0
Organic #5		ACID	N	1.26	33.0	6.50E-02	4.26E-07	ORGCHEM	7.23E+05	8.65E+00	1100	24.2
Organic #5		BASE	N	1.25	30.0	2.70E-02	9.78E-06	ORGCHEM	1.87E+04	5.85E+00	1100	24.2
Organic #5		SOLID	N	1.20	100.0	2.70E+03	.	ORGCHEM	.	1.84E+03	1100	24.2
Organic #5		ACID	Y	1.06	57.0	6.50E-02	3.66E-07	ORGCHEM	7.22E+05	8.65E+00	1100	24.2
Organic #5		BASE	Y	1.04	63.0	2.70E-02	3.66E-07	ORGCHEM	7.22E+05	5.85E+00	1100	24.2
Organic #5		SOLID	Y	1.25	73.0	2.70E+03	7.40E-03	ORGCHEM	1.28E+01	1.84E+03	1100	24.2
Metal #4		ACID	N	1.33	86.0	2.10E-02	8.80E-07	METAL	9.88E+04	2.40E+00	5500	7.8
Metal #4		BASE	N	1.14	88.0	1.60E-02	4.26E-07	METAL	3.92E+05	2.81E+00	5500	7.8
Metal #4		SOLID	N	1.16	37.0	5.24E+03	1.58E-03	METAL	6.10E+00	3.54E+02	5500	7.8
Metal #4		ACID	Y	1.06	86.0	2.10E-02	3.66E-07	METAL	3.92E+05	2.40E+00	5500	7.8
Metal #4		BASE	Y	1.06	93.0	1.60E-02	3.66E-07	METAL	3.92E+05	2.81E+00	5500	7.8
Metal #4		SOLID	Y	1.36	67.0	5.24E+03	1.2041E-01	METAL	3.48E-01	3.54E+02	5500	7.8
Paper #3		ACID	N	1.21	36.0	2.75E+00	4.06E-05	PAPER	5.54E+03	3.44E+00	124010	82.2
Paper #3		BASE	N	1.13	68.0	7.10E-02	4.26E-07	PAPER	3.71E+05	2.07E+01	124010	82.2

Paper #3		SOLID	N	1.15	61.0	1.98E+03	.	PAPER	.	1.12E+02	124010	82.2
Paper #3		ACID	Y	1.67	34.0	2.75E+00	3.66E-07	PAPER	3.71E+05	3.44E+00	124010	82.2
Paper #3		BASE	Y	1.02	79.0	7.10E-02	3.66E-07	PAPER	3.71E+05	2.07E+01	124010	82.2
Paper #3		SOLID	Y	1.10	88.0	1.98E+03	5.20E-04	PAPER	4.35E+01	1.12E+02	124010	82.2
Surface #3	Sample 1	ACID	N	1.21	87.0	5.81E-01	7.08E-05	SURFACE	3.35E+03	2.81E+00	864	11.0
Surface #3	Sample 1	BASE	N	1.07	90.0	4.70E-01	4.26E-07	SURFACE	4.00E+05	3.13E+00	864	11.0
Surface #3	Sample 1	SOLID	N	1.98	87.0	9.29E+03	5.52E-01	SURFACE	3.52E-01	1.96E+02	864	11.0
Surface #3	Sample 1	ACID	Y	1.07	99.0	5.81E-01	3.66E-07	SURFACE	4.00E+05	2.81E+00	864	11.0
Surface #3	Sample 1	BASE	Y	1.06	98.0	4.70E-01	3.66E-07	SURFACE	4.00E+05	3.13E+00	864	11.0
Surface #3	Sample 1	SOLID	Y	1.28	89.0	9.29E+03	4.36E-02	SURFACE	3.15E+00	1.96E+02	864	11.0
Surface #3	Sample 2	ACID	N	1.27	54.0	9.67E+01	1.21E-04	SURFACE	2.25E+03	3.15E+00	2088	64.0
Surface #3	Sample 2	BASE	N	1.28	84.0	6.57E+01	.	SURFACE	.	2.80E+00	2088	64.0
Surface #3	Sample 2	SOLID	N	1.79	7.7	6.23E+04	7.14E-01	SURFACE	1.54E-01	2.11E+02	2088	64.0
Surface #3	Sample 2	ACID	Y	1.05	76.0	9.67E+01	3.66E-07	SURFACE	4.05E+05	3.15E+00	2088	64.0
Surface #3	Sample 2	BASE	Y	1.06	83.0	6.57E+01	3.66E-07	SURFACE	4.05E+05	2.80E+00	2088	64.0
Surface #3	Sample 2	SOLID	Y	2.09	14.0	6.23E+04	2.43E-01	SURFACE	8.25E-01	2.11E+02	2088	64.0
Surface #4		ACID	N	1.19	35.0	3.90E-01	4.26E-07	SURFACE	4.02E+05	1.11E+01	2500	14.8
Surface #4		BASE	N	1.27	59.0	9.40E+01	.	SURFACE	.	1.17E+01	2500	14.8
Surface #4		SOLID	N	1.94	85.0	2.22E+04	2.01E-01	SURFACE	.	1.78E+02	2500	14.8
Surface #4		ACID	Y	1.08	96.0	3.90E-01	3.66E-07	SURFACE	4.02E+05	1.11E+01	2500	14.8
Surface #4		BASE	Y	1.04	80.0	9.40E+01	3.66E-07	SURFACE	4.02E+05	1.17E+01	2500	14.8
Surface #4		SOLID	Y	1.43	97.0	2.22E+04	4.08E-02	SURFACE	2.11E+00	1.78E+02	2500	14.8
Surface #5		ACID	N	1.36	76.0	3.36E+00	5.30E-05	SURFACE	.	3.16E+00	24	9.0
Surface #5		BASE	N	1.22	87.0	3.91E+00	2.24E-05	SURFACE	6.43E+03	3.10E+00	24	9.0
Surface #5		SOLID	N	1.46	60.0	1.45E+05	3.79E-01	SURFACE	2.07E-01	7.80E+02	24	9.0
Surface #5		ACID	Y	1.13	84.0	3.36E+00	3.66E-07	SURFACE	3.72E+05	3.16E+00	24	9.0
Surface #5		BASE	Y	1.03	96.0	3.91E+00	3.66E-07	SURFACE	3.72E+05	3.10E+00	24	9.0
Surface #5		SOLID	Y	1.58	64.0	1.45E+05	8.98E-02	SURFACE	1.58E-01	7.80E+02	24	9.0
Paper #4		ACID	N	1.07	12.5	3.64E-01	4.26E-07	PAPER	4.12E+05	1.78E+01	4030	9.3
Paper #4		BASE	N	1.06	48.0	2.43E-01	4.26E-07	PAPER	4.12E+05	5.00E+00	4030	9.3
Paper #4		SOLID	N	1.12	6.6	1.25E+04	1.58E-03	PAPER	7.67E+01	1.41E+02	4030	9.3
Paper #4		ACID	Y	1.05	27.6	3.64E-01	3.66E-07	PAPER	4.12E+05	1.78E+01	4030	9.3
Paper #4		BASE	Y	1.07	47.4	2.43E-01	3.66E-07	PAPER	4.12E+05	5.00E+00	4030	9.3
Paper #4		SOLID	Y	1.94	35.2	1.25E+04	2.09E-02	PAPER	3.62E+01	1.41E+02	4030	9.3
Petroleum #1	Sample 1	ACID	N	10.40	17.3	2.52E+00	5.47E-05	PETRO	6.14E+03	3.78E+01	18300	33.0
Petroleum #1	Sample 1	BASE	N	1.48	16.3	6.59E-01	2.14E-05	PETRO	8.02E+03	1.01E+01	18300	33.0
Petroleum #1	Sample 1	SOLID	N	1.37	80.0	1.90E+03	1.28E+00	PETRO	1.88E-01	7.35E+01	18300	33.0
Petroleum #1	Sample 1	ACID	Y	6.80	1.6	2.52E+00	5.20E-05	PETRO	1.11E+04	3.78E+01	18300	33.0
Petroleum #1	Sample 1	BASE	Y	1.29	16.0	6.59E-01	1.95E-06	PETRO	4.82E+04	1.01E+01	18300	33.0

Petroleum #1	Sample 1	SOLID	Y	1.76	79.0	1.90E+03	2.68E-02	PETRO	6.80E+00	7.35E+01	18300	33.0
Petroleum #1	Sample 2	ACID	N	1.25	19.6	1.32E+00	4.19E-06	PETRO	2.57E+04	3.46E+00	13000	29.2
Petroleum #1	Sample 2	BASE	N	1.43	57.0	7.44E-01	6.80E-07	PETRO	.	4.38E+00	13000	29.2
Petroleum #1	Sample 2	SOLID	N	1.36	68.0	1.95E+06	.	PETRO	.	5.70E+02	13000	29.2
Petroleum #1	Sample 2	ACID	Y	1.28	31.0	1.32E+00	1.39E-06	PETRO	2.42E+04	3.46E+00	13000	29.2
Petroleum #1	Sample 2	BASE	Y	1.11	61.0	7.44E-01	3.66E-07	PETRO	4.45E+05	4.38E+00	13000	29.2
Petroleum #1	Sample 2	SOLID	Y	1.05	60.0	1.95E+06	5.20E-04	PETRO	2.60E+01	5.70E+02	13000	29.2
Petroleum #2		ACID	N	1.54	25.0	2.62E+00	7.11E-06	PETRO	1.99E+04	1.57E+01	2100	24.0
Petroleum #2		BASE	N	1.42	22.3	7.07E-01	3.03E-06	PETRO	5.37E+04	1.17E+01	2100	24.0
Petroleum #2		SOLID	N	1.42	50.0	5.33E+05	5.44E-01	PETRO	2.01E-01	6.80E+02	2100	24.0
Petroleum #2		ACID	Y	1.31	33.0	2.62E+00	3.65E-05	PETRO	4.29E+03	1.57E+01	2100	24.0
Petroleum #2		BASE	Y	2.51	30.0	7.07E-01	3.32E-06	PETRO	.	1.17E+01	2100	24.0
Petroleum #2		SOLID	Y	1.06	73.0	5.33E+05	5.20E-04	PETRO	1.92E+01	6.80E+02	2100	24.0
Inorganic #4		ACID	N	1.21	88.0	2.20E-01	3.00E-06	INORG	3.20E+04	1.86E+00	14250	4.0
Inorganic #4		BASE	N	1.19	97.0	3.00E-01	1.64E-05	INORG	1.02E+04	1.86E+00	14250	4.0
Inorganic #4		SOLID	N	2.03	68.0	3.62E+03	4.34E+00	INORG	6.07E-02	3.34E+02	14250	4.0
Inorganic #4		ACID	Y	1.03	88.0	2.20E-01	3.66E-07	INORG	4.02E+05	1.86E+00	14250	4.0
Inorganic #4		BASE	Y	1.02	89.0	3.00E-01	3.66E-07	INORG	4.02E+05	1.86E+00	14250	4.0
Inorganic #4		SOLID	Y	1.31	91.0	3.62E+03	1.06E-01	INORG	7.20E-01	3.34E+02	14250	4.0
Metal #5		ACID	N	1.52	85.0	1.80E-02	1.04E-04	METAL	5.95E+02	2.81E+00	180000	1279.0
Metal #5		BASE	N	1.34	100.0	9.00E-03	.	METAL	.	2.49E+00	180000	1279.0
Metal #5		SOLID	N	1.42	51.0	1.10E+02	1.57E-03	METAL	7.89E+01	1.10E+00	180000	1279.0
Metal #5		ACID	Y	1.03	88.0	1.80E-02	3.66E-07	METAL	3.92E+05	2.81E+00	180000	1279.0
Metal #5		BASE	Y	1.13	94.0	9.00E-03	3.66E-07	METAL	3.92E+05	2.49E+00	180000	1279.0
Metal #5		SOLID	Y	1.05	80.0	1.10E+02	5.20E-04	METAL	1.00E+03	1.10E+00	180000	1279.0
Aluminum #5		ACID	N	1.10	93.0	1.40E-01	.	ALUM	.	2.30E+00	744	21.3
Aluminum #5		BASE	N	1.09	89.0	3.90E-02	4.26E-07	ALUM	3.80E+05	2.57E+00	744	21.3
Aluminum #5		SOLID	N	1.43	65.0	4.22E+04	6.91E-01	ALUM	4.33E-01	2.49E+02	744	21.3
Aluminum #5		ACID	Y	1.05	92.0	1.40E-01	3.66E-07	ALUM	3.80E+05	2.30E+00	744	21.3
Aluminum #5		BASE	Y	1.06	94.0	3.90E-02	3.66E-07	ALUM	3.80E+05	2.57E+00	744	21.3
Aluminum #5		SOLID	Y	1.56	75.0	4.22E+04	1.47E-01	ALUM	.	2.49E+02	744	21.3
Aluminum #6	Sample 1	ACID	N	1.25	90.0	9.90E-02	.	ALUM	.	3.40E+00	11450	31.0
Aluminum #6	Sample 1	BASE	N	1.19	85.0	8.90E-02	.	ALUM	.	7.50E+00	11450	31.0
Aluminum #6	Sample 1	SOLID	N	1.70	58.0	1.83E+05	1.68E-01	ALUM	8.50E-01	2.23E+02	11450	31.0
Aluminum #6	Sample 1	ACID	Y	1.04	96.0	9.90E-02	3.66E-07	ALUM	3.25E+05	3.40E+00	11450	31.0
Aluminum #6	Sample 1	BASE	Y	1.07	98.0	8.90E-02	3.66E-07	ALUM	4.00E+05	7.50E+00	11450	31.0
Aluminum #6	Sample 1	SOLID	Y	2.50	48.0	1.83E+05	5.90E-02	ALUM	8.25E-01	2.23E+02	11450	31.0
Aluminum #6	Sample 2	ACID	N	1.36	88.0	1.30E-01	4.26E-07	ALUM	2.61E+05	1.80E+00	5544	8.6
Aluminum #6	Sample 2	BASE	N	1.40	84.0	2.45E-02	1.50E-04	ALUM	1.81E+03	1.40E+00	5544	8.6

Aluminum #6	Sample 2	SOLID	N	1.23	70.0	3.15E+05	.	ALUM	.	1.01E+02	5544	8.6
Aluminum #6	Sample 2	ACID	Y	1.25	83.0	1.30E-01	4.08E-07	ALUM	1.44E+05	1.80E+00	5544	8.6
Aluminum #6	Sample 2	BASE	Y	1.11	92.0	2.45E-02	3.66E-07	ALUM	7.19E+05	1.40E+00	5544	8.6
Aluminum #6	Sample 2	SOLID	Y	1.14	89.0	3.15E+05	.	ALUM	.	1.01E+02	5544	8.6
Paper # 5		ACID	N	1.22	18.0	2.30E-01	8.75E-05	PAPER	5.08E+03	1.20E+01	10802	162.0
Paper # 5		BASE	N	1.30	32.0	1.94E-01	1.63E-04	PAPER	2.49E+03	5.98E+00	10802	162.0
Paper # 5		SOLID	N	1.68	59.0	1.43E+04	4.91E-03	PAPER	.	1.15E+02	10802	162.0
Paper # 5		ACID	Y	1.05	56.0	2.30E-01	3.66E-07	PAPER	4.10E+05	1.20E+01	10802	162.0
Paper # 5		BASE	Y	1.09	82.0	1.94E-01	3.66E-07	PAPER	4.10E+05	5.98E+00	10802	162.0
Paper # 5		SOLID	Y	1.30	62.0	1.43E+04	1.14E-02	PAPER	1.76E+00	1.15E+02	10802	162.0
Aluminum #2		ACID	N	1.21	79.0	3.00E-02	6.35E-06	ALUM	1.95E+04	9.12E-01	5544	0.6
Aluminum #2		BASE	N	1.24	88.0	1.40E-02	1.56E-05	ALUM	9.40E+03	1.21E+00	5544	0.6
Aluminum #2		SOLID	N	1.74	94.0	7.64E+04	6.59E+00	ALUM	2.13E-02	1.38E+03	5544	0.6
Aluminum #2		ACID	Y	1.10	85.0	3.00E-02	3.66E-07	ALUM	7.67E+05	9.12E-01	5544	0.6
Aluminum #2		BASE	Y	1.04	100.0	1.40E-02	3.66E-07	ALUM	7.67E+05	1.21E+00	5544	0.6
Aluminum #2		SOLID	Y	1.25	93.0	7.64E+04	5.87E-01	ALUM	2.84E-01	1.38E+03	5544	0.6
Petroleum #3		ACID	N	1.16	90.0	9.54E-01	1.90E-06	PETRO	6.82E+04	4.60E+00	10426	16.8
Petroleum #3		BASE	N	1.16	35.0	2.50E-01	4.26E-07	PETRO	4.28E+05	3.23E+00	10426	16.8
Petroleum #3		SOLID	N	1.53	90.0	8.03E+05	6.45E-01	PETRO	5.10E-01	1.55E+02	10426	16.8
Petroleum #3		ACID	Y	1.06	58.0	9.54E-01	3.66E-07	PETRO	4.28E+05	4.60E+00	10426	16.8
Petroleum #3		BASE	Y	1.11	62.0	2.50E-01	4.60E-07	PETRO	3.12E+05	3.23E+00	10426	16.8
Petroleum #3		SOLID	Y	1.08	76.0	8.03E+05	.	PETRO	.	1.55E+02	10426	16.8
Metal #6		ACID	N	1.33	97.0	1.37E-01	1.67E-05	METAL	8.00E+03	2.02E+00	45300	23.0
Metal #6		BASE	N	1.41	82.0	1.38E-01	9.30E-05	METAL	2.65E+03	2.08E+00	45300	23.0
Metal #6		SOLID	N	1.66	58.0	1.52E+04	1.32E+00	METAL	3.43E-01	2.47E+02	45300	23.0
Metal #6		ACID	Y	1.02	96.0	1.37E-01	3.66E-07	METAL	3.72E+05	2.02E+00	45300	23.0
Metal #6		BASE	Y	1.06	100.0	1.38E-01	3.66E-07	METAL	3.72E+05	2.08E+00	45300	23.0
Metal #6		SOLID	Y	1.15	52.0	1.52E+04	1.53E-02	METAL	5.04E+00	2.47E+02	45300	23.0
Paper #6		ACID	N	10.50	9.0	2.50E-01	1.53E-04	PAPER	1.10E+03	2.83E+01	77000	1484.0
Paper #6		BASE	N	2.13	13.5	1.71E-01	5.48E-06	PAPER	.	4.80E+01	77000	1484.0
Paper #6		SOLID	N	1.04	76.0	1.38E+03	1.58E-03	PAPER	3.60E+02	7.00E+01	77000	1484.0
Paper #6		ACID	Y	5.70	11.0	2.50E-01	6.00E-05	PAPER	5.33E+03	2.83E+01	77000	1484.0
Paper #6		BASE	Y	1.80	24.5	1.71E-01	2.05E-06	PAPER	2.71E+04	4.80E+01	77000	1484.0
Paper #6		SOLID	Y	1.60	61.0	1.38E+03	5.20E-04	PAPER	2.76E+01	7.00E+01	77000	1484.0
Inorganic #5	Sample 1	ACID	N	1.18	79.0	4.30E-02	.	INORG	.	5.20E+00	8400	259.3
Inorganic #5	Sample 1	BASE	N	1.14	92.0	6.50E-02	.	INORG	.	2.37E+00	8400	259.3
Inorganic #5	Sample 1	SOLID	N	1.22	60.0	2.27E+02	3.63E-02	INORG	.	8.79E+00	8400	259.3
Inorganic #5	Sample 1	ACID	Y	1.02	84.0	4.30E-02	3.66E-07	INORG	4.12E+05	5.20E+00	8400	259.3
Inorganic #5	Sample 1	BASE	Y	1.12	87.0	6.50E-02	3.66E-07	INORG	4.12E+05	2.37E+00	8400	259.3

Inorganic #5	Sample 1	SOLID	Y	1.04	85.0	2.27E+02	5.20E-04	INORG	2.14E+02	8.79E+00	8400	259.3
Inorganic #5	Sample 2	ACID	N	1.22	90.0	4.00E-02	2.80E-05	INORG	1.22E+04	2.50E+00	8400	199.0
Inorganic #5	Sample 2	BASE	N	1.25	77.0	4.50E-02	.	INORG	.	2.05E+00	8400	199.0
Inorganic #5	Sample 2	SOLID	N	2.10	54.0	3.90E+01	3.99E-02	INORG	2.83E+00	4.40E+00	8400	199.0
Inorganic #5	Sample 2	ACID	Y	1.07	91.0	4.00E-02	3.66E-07	INORG	4.14E+05	2.50E+00	8400	199.0
Inorganic #5	Sample 2	BASE	Y	1.05	100.0	4.50E-02	3.66E-07	INORG	4.14E+05	2.05E+00	8400	199.0
Inorganic #5	Sample 2	SOLID	Y	1.32	81.0	3.90E+01	2.40E-03	INORG	5.07E+01	4.40E+00	8400	199.0
Petroleum #4	Sample 1	ACID	N	1.84	59.0	6.64E-01	5.22E-06	PETRO	1.28E+04	6.56E+00	9456	32.0
Petroleum #4	Sample 1	BASE	N	1.31	76.0	2.80E-01	.	PETRO	.	2.81E+00	9456	32.0
Petroleum #4	Sample 1	SOLID	N	1.90	45.0	1.78E+06	1.84E+00	PETRO	4.00E-02	3.41E+03	9456	32.0
Petroleum #4	Sample 1	ACID	Y	1.51	64.0	6.64E-01	1.07E-06	PETRO	8.75E+04	6.56E+00	9456	32.0
Petroleum #4	Sample 1	BASE	Y	1.08	78.0	2.80E-01	3.66E-07	PETRO	4.00E+05	2.81E+00	9456	32.0
Petroleum #4	Sample 1	SOLID	Y	1.28	59.0	1.78E+06	1.45E-01	PETRO	7.14E-01	3.41E+03	9456	32.0
Petroleum #4	Sample 2	ACID	N	1.33	51.0	1.01E+00	9.96E-06	PETRO	1.96E+04	4.06E+00	9456	32.0
Petroleum #4	Sample 2	BASE	N	1.18	78.0	1.28E-01	.	PETRO	.	2.56E+00	9456	32.0
Petroleum #4	Sample 2	SOLID	N	1.34	77.0	7.43E+05	.	PETRO	.	1.39E+03	9456	32.0
Petroleum #4	Sample 2	ACID	Y	1.40	60.0	1.01E+00	2.01E-06	PETRO	7.33E+04	4.06E+00	9456	32.0
Petroleum #4	Sample 2	BASE	Y	1.07	89.0	1.28E-01	3.66E-07	PETRO	4.00E+05	2.56E+00	9456	32.0
Petroleum #4	Sample 2	SOLID	Y	1.24	74.0	7.43E+05	8.52E-02	PETRO	8.35E-01	1.39E+03	9456	32.0
Organic #6	Sample 1	ACID	N	1.35	88.0	3.50E-01	.	ORGCHEM	.	8.50E+00	4752	29.0
Organic #6	Sample 1	BASE	N	1.34	80.0	1.30E-01	4.26E-07	ORGCHEM	4.06E+05	5.17E+00	4752	29.0
Organic #6	Sample 1	SOLID	N	1.51	74.0	1.81E+03	1.58E-03	ORGCHEM	2.36E+01	7.65E+00	4752	29.0
Organic #6	Sample 1	ACID	Y	1.05	89.0	3.50E-01	3.66E-07	ORGCHEM	4.06E+05	8.50E+00	4752	29.0
Organic #6	Sample 1	BASE	Y	1.08	92.0	1.30E-01	3.66E-07	ORGCHEM	4.06E+05	5.17E+00	4752	29.0
Organic #6	Sample 1	SOLID	Y	1.10	83.0	1.81E+03	1.20E-02	ORGCHEM	1.86E+01	7.65E+00	4752	29.0
Organic #6	Sample 2	ACID	N	1.18	63.0	2.90E-01	.	ORGCHEM	.	8.94E+00	4752	29.2
Organic #6	Sample 2	BASE	N	1.50	83.0	1.80E-01	.	ORGCHEM	.	4.75E+00	4752	29.2
Organic #6	Sample 2	SOLID	N	1.91	19.0	2.30E+04	4.78E+00	ORGCHEM	2.71E-02	3.46E+02	4752	29.2
Organic #6	Sample 2	ACID	Y	1.07	95.0	2.90E-01	3.66E-07	ORGCHEM	4.00E+05	8.94E+00	4752	29.2
Organic #6	Sample 2	BASE	Y	1.06	87.0	1.80E-01	3.66E-07	ORGCHEM	4.00E+05	4.75E+00	4752	29.2
Organic #6	Sample 2	SOLID	Y	2.80	34.0	2.30E+04	6.92E-02	ORGCHEM	.	3.46E+02	4752	29.2

C) Data used to generate Figure 7 and Table 3 of Chapter 3.

Variables: as above in B) in addition to:

BaP Load = Loading in g Benzo(a)pyrene genotoxic equivalents per day.

Geno. Load = Genotoxic Loading in SOS Induction Factor units per Day.

(Note = 2.52×10^9 IF per day = 1 kg BaP Equivalents per day).

Industry	Sub-Samp	Sample Type	Ind. Type	BaP Load	Geno. Load
Aluminum #1		ACID	ALUM	2.926E-01	7.373E+05
Aluminum #1		BASE	ALUM	.	.
Aluminum #1		SOLID	ALUM	1.932E+00	4.870E+06
Paper #1		ACID	PAPER	9.855E+01	2.483E+08
Paper #1		BASE	PAPER	2.675E+03	6.740E+09
Paper #1		SOLID	PAPER	.	.
Inorganic #1		ACID	INORG	6.016E+01	1.516E+08
Inorganic #1		BASE	INORG	1.126E+01	2.838E+07
Inorganic #1		SOLID	INORG	9.160E+00	2.308E+07
Aluminum #3		ACID	ALUM	1.817E+01	4.579E+07
Aluminum #3		BASE	ALUM	.	.
Aluminum #3		SOLID	ALUM	1.810E+01	4.562E+07
Aluminum #1	Sample 1	ACID	ALUM	.	.
Aluminum #1	Sample 1	BASE	ALUM	1.096E+02	2.762E+08
Aluminum #1	Sample 1	SOLID	ALUM	4.343E+00	1.094E+07
Aluminum #4		ACID	ALUM	3.869E-01	9.750E+05
Aluminum #4		BASE	ALUM	1.151E+00	2.900E+06
Aluminum #4		SOLID	ALUM	9.866E-01	2.486E+06
Organic #1		ACID	ORGCHEM	.	.
Organic #1		BASE	ORGCHEM	1.267E+01	3.194E+07
Organic #1		SOLID	ORGCHEM	1.057E+01	2.663E+07
Metal #1		ACID	METAL	2.144E+03	5.403E+09
Metal #1		BASE	METAL	.	.
Metal #1		SOLID	METAL	3.088E+01	7.781E+07
Metal #2		BASE	METAL	3.124E+02	7.872E+08
Metal #2		SOLID	METAL	8.866E+00	2.234E+07
Metal #3	Sample 1	ACID	METAL	2.012E+03	5.069E+09
Metal #3	Sample 1	SOLID	METAL	9.646E+00	2.431E+07
Metal #3	Sample 2	ACID	METAL	.	.
Metal #3	Sample 2	SOLID	METAL	2.894E+01	7.293E+07
Sewage #1		ACID	SEWAGE	4.966E+02	1.251E+09
Sewage #1		BASE	SEWAGE	3.026E+04	7.626E+10
Sewage #1		SOLID	SEWAGE	3.485E+02	8.781E+08
Paper #2		ACID	PAPER	1.711E+02	4.312E+08

Paper #2		BASE	PAPER	2.352E+01	5.928E+07
Paper #2		SOLID	PAPER	6.027E+01	1.519E+08
Organic #7		ACID	ORGCHEM	7.661E+01	1.931E+08
Organic #7		BASE	ORGCHEM	9.696E+01	2.443E+08
Organic #7		SOLID	ORGCHEM	1.931E+02	4.867E+08
Surface #1		ACID	SURFACE	3.564E-01	8.982E+05
Surface #1		BASE	SURFACE	1.234E-01	3.111E+05
Surface #1		SOLID	SURFACE	6.310E-01	1.590E+06
Organic #2		ACID	ORGCHEM	4.371E+01	1.102E+08
Organic #2		BASE	ORGCHEM	4.842E+01	1.220E+08
Organic #2		SOLID	ORGCHEM	1.024E+01	2.582E+07
Inorganic #2		ACID	INORG	8.381E+01	2.112E+08
Inorganic #2		SOLID	INORG	9.673E+00	2.438E+07
Organic #3		ACID	ORGCHEM	5.238E-02	1.320E+05
Organic #3		SOLID	ORGCHEM	5.903E-02	1.488E+05
Inorganic #3	Sample 1	ACID	INORG	.	.
Inorganic #3	Sample 1	BASE	INORG	.	.
Inorganic #3	Sample 1	SOLID	INORG	.	.
Inorganic #3	Sample 2	ACID	INORG	.	.
Inorganic #3	Sample 2	SOLID	INORG	.	.
Surface #2		ACID	SURFACE	.	.
Surface #2		BASE	SURFACE	.	.
Surface #2		SOLID	SURFACE	1.270E-01	3.199E+05
Organic #4		ACID	ORGCHEM	.	.
Organic #4		BASE	ORGCHEM	.	.
Organic #4		SOLID	ORGCHEM	.	.
Organic #5		BASE	ORGCHEM	4.269E+00	1.076E+07
Organic #5		SOLID	ORGCHEM	7.817E-02	1.970E+05
Metal #4		ACID	METAL	1.921E+00	4.840E+06
Metal #4		SOLID	METAL	2.037E+00	5.133E+06
Paper #3		ACID	PAPER	1.998E+03	5.034E+09
Paper #3		SOLID	PAPER	.	.
Surface #3	Sample 1	ACID	SURFACE	2.427E+01	6.117E+07
Surface #3	Sample 1	SOLID	SURFACE	2.080E+00	5.242E+06
Surface #3	Sample 2	ACID	SURFACE	1.000E+02	2.520E+08
Surface #3	Sample 2	BASE	SURFACE	.	.
Surface #3	Sample 2	SOLID	SURFACE	3.788E+01	9.545E+07
Surface #4		BASE	SURFACE	.	.
Surface #4		SOLID	SURFACE	2.957E+00	7.452E+06

Surface #5		ACID	SURFACE	5.048E-01	1.272E+06
Surface #5		BASE	SURFACE	2.137E-01	5.386E+05
Surface #5		SOLID	SURFACE	3.249E-02	8.187E+04
Paper #4		SOLID	PAPER	3.102E-01	7.818E+05
Petroleum #1	Sample 1	ACID	PETRO	3.970E+02	1.000E+09
Petroleum #1	Sample 1	BASE	PETRO	1.551E+02	3.908E+08
Petroleum #1	Sample 1	SOLID	PETRO	3.063E+02	7.718E+08
Petroleum #1	Sample 2	ACID	PETRO	2.161E+01	5.446E+07
Petroleum #1	Sample 2	BASE	PETRO	3.508E+00	8.840E+06
Petroleum #1	Sample 2	SOLID	PETRO	.	.
Petroleum #2		ACID	PETRO	3.045E+01	7.673E+07
Petroleum #2		BASE	PETRO	2.767E+00	6.972E+06
Petroleum #2		SOLID	PETRO	1.088E+01	2.742E+07
Inorganic #4		ACID	INORG	1.696E+01	4.275E+07
Inorganic #4		BASE	INORG	9.274E+01	2.337E+08
Inorganic #4		SOLID	INORG	9.811E+01	2.473E+08
Metal #5		ACID	METAL	7.429E+03	1.872E+10
Metal #5		BASE	METAL	.	.
Metal #5		SOLID	METAL	1.436E+02	3.618E+08
Aluminum #6	Sample 2	ACID	ALUM	8.967E-01	2.260E+06
Aluminum #6	Sample 2	BASE	ALUM	3.300E+02	8.316E+08
Aluminum #6	Sample 2	SOLID	ALUM	.	.
Aluminum #6	Sample 1	ACID	ALUM	.	.
Aluminum #6	Sample 1	BASE	ALUM	.	.
Aluminum #6	Sample 1	SOLID	ALUM	2.362E+01	5.952E+07
Aluminum #5		ACID	ALUM	.	.
Aluminum #5		SOLID	ALUM	4.347E+00	1.095E+07
Paper #5		ACID	PAPER	3.751E+02	9.452E+08
Paper #5		BASE	PAPER	6.974E+02	1.758E+09
Paper #5		SOLID	PAPER	7.916E+00	1.995E+07
Aluminum #2	Sample 2	ACID	ALUM	1.398E+01	3.522E+07
Aluminum #2	Sample 2	BASE	ALUM	3.442E+01	8.674E+07
Aluminum #2	Sample 2	SOLID	ALUM	8.553E+00	2.155E+07
Petroleum #3		ACID	PETRO	7.861E+00	1.981E+07
Petroleum #3		BASE	PETRO	1.903E+00	4.796E+06
Petroleum #3		SOLID	PETRO	4.481E+01	1.129E+08
Metal #6		ACID	METAL	3.010E+02	7.585E+08
Metal #6		BASE	METAL	1.672E+03	4.214E+09
Metal #6		SOLID	METAL	5.469E+02	1.378E+09

Paper #6		ACID	PAPER	4.687E+03	1.181E+10
Paper #6		BASE	PAPER	1.674E+02	4.220E+08
Inorganic #5	Sample 1	ACID	INORG	.	.
Inorganic #5	Sample 1	BASE	INORG	.	.
Inorganic #5	Sample 1	SOLID	INORG	3.133E+01	7.896E+07
Inorganic #5	Sample 2	ACID	INORG	9.333E+01	2.352E+08
Inorganic #5	Sample 2	BASE	INORG	.	.
Inorganic #5	Sample 2	SOLID	INORG	2.650E+01	6.677E+07
Petroleum #4	Sample 2	ACID	PETRO	3.739E+01	9.422E+07
Petroleum #4	Sample 2	BASE	PETRO	.	.
Petroleum #4	Sample 2	SOLID	PETRO	1.023E+01	2.578E+07
Petroleum #4	Sample 1	ACID	PETRO	1.957E+01	4.932E+07
Petroleum #4	Sample 1	BASE	PETRO	.	.
Petroleum #4	Sample 1	SOLID	PETRO	2.206E+02	5.558E+08
Organic #6	Sample 1	ACID	ORGCHEM	.	.
Organic #6	Sample 1	SOLID	ORGCHEM	6.562E-01	1.654E+06
Organic #6	Sample 2	ACID	ORGCHEM	.	.
Organic #6	Sample 2	BASE	ORGCHEM	.	.
Organic #6	Sample 2	SOLID	ORGCHEM	2.615E+02	6.591E+08

C) Data used to generate Table 4 of Chapter 3.

Variables: as above in B) and C) in addition to:

Pred Mut. Pot = Predicted *Ames* mutagenic potency in Revertents per mg Residue per assay mL (top agar).

Predictions are maximum mutagenic potency values calculated using models 5 and 6 in Chapter 2.

Industry	Sub-Sample	Activ.	Sample Type	Pred. Mut. Pot.
Aluminum #1		N	ACID	922.18
Aluminum #1		N	BASE	.
Aluminum #1		N	SOLID	3177.60
Aluminum #1		Y	SOLID	181.00
Paper #1		N	ACID	3064.20
Paper #1		N	BASE	17097.00
Paper #1		N	SOLID	.
Inorganic #1		N	ACID	19810.00

Inorganic #1		N	BASE	4515.00
Inorganic #1		N	SOLID	14340.00
Inorganic #1		Y	ACID	2389.80
Inorganic #1		Y	SOLID	10959.00
Aluminum #3		N	ACID	1498.10
Aluminum #3		N	BASE	.
Aluminum #3		N	SOLID	2014.50
Aluminum #3		Y	ACID	451.70
Aluminum #3		Y	SOLID	1609.40
Aluminum #2	Sample 1	N	ACID	.
Aluminum #2	Sample 1	N	BASE	51507.00
Aluminum #2	Sample 1	N	SOLID	5197.60
Aluminum #2	Sample 1	Y	SOLID	3470.70
Aluminum #4		N	ACID	6712.60
Aluminum #4		N	BASE	13413.00
Aluminum #4		N	SOLID	15000.00
Aluminum #4		Y	BASE	1025.10
Aluminum #4		Y	SOLID	2577.80
Organic #1		N	BASE	16690.00
Organic #1		N	SOLID	20170.00
Organic #1		Y	ACID	.
Organic #1		Y	BASE	32854.00
Organic #1		Y	SOLID	895.97
Metal #1		N	ACID	47264.00
Metal #1		N	BASE	.
Metal #1		N	SOLID	1088.60
Metal #1		Y	BASE	.
Metal #1		Y	SOLID	309.33
Metal #2		N	BASE	6479.20
Metal #2		N	SOLID	1083.20
Metal #2		Y	SOLID	1571.40
Metal #3	Sample 1	N	ACID	19654.00
Metal #3	Sample 1	N	SOLID	.
Metal #3	Sample 1	Y	ACID	.
Metal #3	Sample 1	Y	SOLID	8.76
Metal #3	Sample 2	N	ACID	.
Metal #3	Sample 2	N	SOLID	1822.50
Metal #3	Sample 2	Y	SOLID	5714.40
Sewage #1		N	ACID	.

Sewage #1		N	BASE	38045.00
Sewage #1		N	SOLID	915.26
Sewage #1		Y	ACID	2173.20
Sewage #1		Y	BASE	.
Sewage #1		Y	SOLID	476.48
Paper #2		N	ACID	587.86
Paper #2		N	BASE	405.50
Paper #2		Y	ACID	4078.80
Paper #2		Y	BASE	631.16
Paper #2		Y	SOLID	1206.50
Organic #7		N	ACID	6620.10
Organic #7		N	BASE	7372.40
Organic #7		N	SOLID	47949.00
Organic #7		Y	ACID	.
Organic #7		Y	BASE	1899.40
Organic #7		Y	SOLID	1564.90
Surface #1		N	ACID	1078.80
Surface #1		N	BASE	.
Surface #1		N	SOLID	2065.80
Surface #1		Y	BASE	695.32
Surface #1		Y	SOLID	895.55
Organic #2		N	ACID	28925.00
Organic #2		N	BASE	24497.00
Organic #2		N	SOLID	183550.00
Organic #2		Y	SOLID	31051.00
Inorganic #2		N	ACID	27502.00
Inorganic #2		N	SOLID	1695.50
Inorganic #2		Y	SOLID	562.53
Organic #3		N	ACID	7711.10
Organic #3		N	SOLID	12029.00
Organic #3		Y	SOLID	1207.60
Inorganic #3	Sample 1	N	ACID	.
Inorganic #3	Sample 1	N	BASE	9665.90
Inorganic #3	Sample 1	N	SOLID	38974.00
Inorganic #3	Sample 1	Y	BASE	.
Inorganic #3	Sample 1	Y	SOLID	3011.00
Inorganic #3	Sample 2	N	ACID	48176.00
Inorganic #3	Sample 2	N	SOLID	10525.00
Inorganic #3	Sample 2	Y	SOLID	.

Surface #2		N	ACID	.
Surface #2		N	BASE	.
Surface #2		N	SOLID	7901.60
Surface #2		Y	SOLID	746.00
Organic #4		N	ACID	.
Organic #4		N	BASE	.
Organic #4		N	SOLID	.
Organic #4		Y	ACID	.
Organic #4		Y	BASE	.
Organic #4		Y	SOLID	.
Organic #5		N	BASE	5123.30
Organic #5		N	SOLID	.
Organic #5		Y	SOLID	28.63
Metal #4		N	ACID	1569.00
Metal #4		Y	SOLID	1851.80
Paper #3		N	ACID	23528.00
Paper #3		N	SOLID	.
Surface #3	Sample 1	N	ACID	42514.00
Surface #3	Sample 1	N	SOLID	7690.80
Surface #3	Sample 1	Y	SOLID	1241.90
Surface #3	Sample 2	N	ACID	58954.00
Surface #3	Sample 2	N	BASE	.
Surface #3	Sample 2	N	SOLID	8876.80
Surface #3	Sample 2	Y	SOLID	5817.30
Surface #4		N	BASE	.
Surface #4		N	SOLID	3775.30
Surface #4		Y	SOLID	1276.10
Surface #5		N	ACID	30951.00
Surface #5		N	BASE	16072.00
Surface #5		N	SOLID	1954.80
Surface #5		Y	SOLID	668.54
Paper #4		Y	SOLID	847.00
Petroleum #1	Sample 1	N	ACID	4575.60
Petroleum #1	Sample 1	N	BASE	6172.60
Petroleum #1	Sample 1	N	SOLID	31834.00
Petroleum #1	Sample 1	Y	ACID	6885.00
Petroleum #1	Sample 1	Y	BASE	1088.80
Petroleum #1	Sample 1	Y	SOLID	1976.20
Petroleum #1	Sample 2	N	ACID	3983.60

Petroleum #1	Sample 2	N	BASE	802.61
Petroleum #1	Sample 2	N	SOLID	.
Petroleum #1	Sample 2	Y	ACID	2170.60
Petroleum #2		N	ACID	1849.40
Petroleum #2		N	BASE	1195.20
Petroleum #2		N	SOLID	2884.40
Petroleum #2		Y	ACID	11286.00
Petroleum #2		Y	BASE	1561.30
Inorganic #4		N	ACID	4982.30
Inorganic #4		N	BASE	18744.00
Inorganic #4		N	SOLID	25353.00
Inorganic #4		Y	SOLID	1734.50
Metal #5		N	ACID	57384.00
Metal #5		N	BASE	.
Metal #5		N	SOLID	4542.00
Aluminum #6	Sample 2	N	BASE	131480.00
Aluminum #6	Sample 2	N	SOLID	.
Aluminum #6	Sample 2	Y	ACID	1262.90
Aluminum #6	Sample 2	Y	SOLID	.
Aluminum #6	Sample 1	N	ACID	.
Aluminum #6	Sample 1	N	BASE	.
Aluminum #6	Sample 1	N	SOLID	2749.40
Aluminum #6	Sample 1	Y	SOLID	1463.10
Aluminum #5		N	ACID	.
Aluminum #5		N	SOLID	7614.00
Aluminum #5		Y	SOLID	3101.20
Paper #5		N	ACID	16215.00
Paper #5		N	BASE	45139.00
Paper #5		N	SOLID	293.76
Paper #5		Y	SOLID	582.38
Aluminum #2	Sample 2	N	ACID	15598.00
Aluminum #2	Sample 2	N	BASE	25268.00
Aluminum #2	Sample 2	N	SOLID	11610.00
Aluminum #2	Sample 2	Y	SOLID	2283.80
Petroleum #3		N	ACID	1721.80
Petroleum #3		N	SOLID	10434.00
Petroleum #3		Y	BASE	816.67
Petroleum #3		Y	SOLID	.
Metal #6		N	ACID	17861.00

Metal #6		N	BASE	66513.00
Metal #6		N	SOLID	12716.00
Metal #6		Y	SOLID	374.95
Paper #6		N	ACID	12824.00
Paper #6		N	BASE	631.51
Paper #6		Y	ACID	10339.00
Paper #6		Y	BASE	262.98
Inorganic #5	Sample 1	N	ACID	.
Inorganic #5	Sample 1	N	BASE	.
Inorganic #5	Sample 1	N	SOLID	10364.00
Inorganic #5	Sample 2	N	ACID	22588.00
Inorganic #5	Sample 2	N	BASE	.
Inorganic #5	Sample 2	N	SOLID	19175.00
Inorganic #5	Sample 2	Y	SOLID	2885.70
Petroleum #4	Sample 2	N	ACID	6912.20
Petroleum #4	Sample 2	N	BASE	.
Petroleum #4	Sample 2	N	SOLID	.
Petroleum #4	Sample 2	Y	ACID	2629.70
Petroleum #4	Sample 2	Y	SOLID	369.42
Petroleum #4	Sample 1	N	ACID	2869.40
Petroleum #4	Sample 1	N	BASE	.
Petroleum #4	Sample 1	N	SOLID	2116.00
Petroleum #4	Sample 1	Y	ACID	931.14
Petroleum #4	Sample 1	Y	SOLID	261.38
Organic #6	Sample 1	N	ACID	.
Organic #6	Sample 1	Y	SOLID	7789.20
Organic #6	Sample 2	N	ACID	.
Organic #6	Sample 2	N	BASE	.
Organic #6	Sample 2	N	SOLID	26617.00
Organic #6	Sample 2	Y	SOLID	1123.80

D) Data used (in addition to data above in B) to generate Figure 3 of Chapter 4.

Variables:

K_d-GENO = Log Sorption partition coefficient of genotoxicity.
Calculated as described in Chapter 4.

K_d-FLUOR = Log Sorption partition coefficient of fluorescence.
Calculated as described in Chapter 4.

Industry	Sub-Sample	K _d -GENO	K _d -FLUOR
Aluminum #1		4.88	5.71
Paper #1		3.93	4.62
Inorganic #1		6.04	4.44
Aluminum #3		6.82	6.92
Aluminum #2	Sample 1	.	7.08
Aluminum #4		5.77	5.74
Organic #1		5.48	5.42
Sewage #2		3.91	3.83
Metal #1		3.80	6.23
Metal #2		6.33	5.00
Metal #3	Sample 1	3.71	5.93
Metal #3	Sample 2	6.71	4.79
Sewage #1		3.88	4.08
Paper #2		3.36	3.38
Organic #7		4.75	5.48
Surface #1		.	5.61
Organic #2		5.29	4.92
Inorganic #2		6.39	5.51
Organic #3		6.02	6.59
Inorganic #3	Sample 1	5.02	4.82
Inorganic #3	Sample 2	5.40	4.71
Surface #2		4.46	4.53
Organic #4		.	4.70
Organic #5		4.63	4.85
Metal #4		4.99	5.46
Paper #3		3.81	4.16
Surface #3	Sample 1	5.23	4.25
Surface #3	Sample 2	5.79	2.90
Surface #4		5.28	4.46
Surface #5		6.19	4.60
Paper #4		3.86	4.63
Petroleum #1	Sample 1	3.72	3.26
Petroleum #1	Sample 2	3.96	6.31
Petroleum #2		3.53	5.68
Inorganic #4		5.73	4.15

Metal #5		2.74	3.96
Aluminum #6	Sample 1	.	6.88
Aluminum #6	Sample 2	5.64	6.29
Aluminum #5		5.94	5.84
Paper #5		5.07	4.83
Aluminum #2	Sample 2	6.41	6.60
Petroleum #3		5.78	6.31
Metal #6		4.85	5.04
Paper #6		1.89	3.83
Inorganic #5	Sample 1	3.29	3.64
Inorganic #5	Sample 2	4.07	2.96
Petroleum #4	Sample 2	5.43	6.51
Petroleum #4	Sample 1	5.78	6.65
Organic #6	Sample 1	4.28	3.98
Organic #6	Sample 2	7.17	5.02

APPENDIX 7

Data Used to Generate Figures and Tables in Chapter 5

1) Data used to calculate Genotoxicity Equivalency Factors listed in Table 3, Chapter 5. Data sources are provided in the footnote of Table 3, Chapter 5 in addition to Appendix 5.

Variables:

SOSIP or SRIP = SOS Chromotest Genotoxic Potency in IF Units per μg per assay mL

GEF = Genotoxicity Equivalency Factor - Potency relative to Benzo(a)pyrene

Compound	SOSIP or SRIP	GEF
3-methylcholanthrene	0.467	0.215
7,12-dimethylbenz(a)anthracene	0.451	0.208
Anthracene	0.072	0.033
Benzo(a)pyrene	2.168	1.000
Benzo(c)phenanthrene	3.250	1.499
Benzo(ghi)perylene	0.054	0.025
Benzo(j)fluoranthene	0.568	0.262
Chrysene	0.225	0.104
Dibenz(a,h)anthracene	0.083	0.038
Dibenz(a,i)pyrene	0.494	0.228
Dibenz(a,l)pyrene	2.575	1.188
Dibenz(a,h)pyrene	0.083	0.038
Indeno(1,2,3-cd)pyrene	0.061	0.028
Fluoranthene	0.864	0.399
Phenanthrene	0.218	0.101
Pyrene	0.116	0.053
Benzo(a)anthracene	0.046	0.021

2) Concentration-response data used to generate Figure 1, Chapter 5.

Variables:

Sample = BSed - Bottom Sediment, SSed - Suspended Particulate Matter

Concentration = g dry sediment per assay mL

Activ = Activation Status

Site	Sample	Concentration	Mean IF	Std. Error	Activ.
Jonquière	BSed	2.91E-02	1.02	6.26E-03	N
Jonquière	BSed	5.82E-02	1.05	2.28E-02	N
Jonquière	BSed	1.16E-01	1.08	7.26E-03	N
Jonquière	BSed	2.33E-01	1.10	2.90E-04	N
Jonquière	BSed	4.65E-01	1.09	4.95E-02	N
Jonquière	BSed	9.31E-01	0.80	1.00E-01	N
Jonquière	BSed	2.91E-02	1.21	8.13E-02	Y
Jonquière	BSed	5.82E-02	1.49	5.11E-02	Y
Jonquière	BSed	1.16E-01	1.60	1.22E-01	Y
Jonquière	BSed	2.33E-01	1.64	1.16E-01	Y
Jonquière	BSed	4.65E-01	1.46	9.57E-02	Y
Jonquière	BSed	9.31E-01	1.43	1.27E-01	Y
Jonquière	SSed	1.45E-02	1.26	8.17E-02	N
Jonquière	SSed	2.90E-02	1.48	4.41E-02	N
Jonquière	SSed	5.80E-02	1.72	3.70E-02	N
Jonquière	SSed	1.16E-01	1.94	7.41E-02	N
Jonquière	SSed	2.32E-01	1.90	1.10E-01	N
Jonquière	SSed	4.64E-01	2.16	1.22E-01	N
Jonquière	SSed	2.90E-02	1.20	5.14E-02	Y
Jonquière	SSed	5.80E-02	1.18	1.10E-02	Y
Jonquière	SSed	1.16E-01	1.19	2.31E-02	Y
Jonquière	SSed	2.32E-01	1.21	8.11E-02	Y
Jonquière	SSed	4.64E-01	1.25	1.03E-01	Y
Jonquière	SSed	9.28E-01	1.62	2.02E-01	Y

3) Bottom sediment genotoxicity results used in Figures 6, 7 and 8 of Chapter 5.

Variables:

Extracted = Quantity of sediment extracted and subjected to GPC clean-up in grams dry wt.

Water = Water content - fraction of wet sediment.

Org. = Organic matter content - Loss on ignition at 550 °C, fraction of dry sediment.

Response = SOS Chromotest result. (M/Erratic = Marginal/Erratic).

Above = Number of concentrations that exceed upper confidence limit of control.

Activ. = S9 Activation status.

SRIP = SOS Response Inducing Potency in IF Units per g dry sediment per assay mL.

LOGC = Lowest Observable Genotoxic Concentration in g dry sediment.

MaxIF = Maximum Induction Factor Observed.

Site	Extracted	Water	Org.	Response	Above	Activ	SRIP-Dry	LOGC-Dry	MaxIF
Alma	49.70	0.4477	0.0757	NEG	0	N	.	.	0.99
Alma	49.70	0.4477	0.0757	NEG	0	Y	.	.	1.01
Beauharnois	49.92	0.7771	0.1039	NEG	0	N	.	.	0.88
Beauharnois	49.92	0.7771	0.1039	NEG	0	Y	.	.	0.99
Beauport	50.00	0.2795	0.0071	M/Erratic	1	N	.	.	1.04
Beauport	50.00	0.2795	0.0071	MARG	1	Y	1.10E-01	5.08E-01	1.04
Beaupré	50.26	0.5294	0.0354	NEG	0	N	.	.	0.90
Beaupré	50.26	0.5294	0.0354	MARG	2	Y	4.00E-01	1.13E-01	1.07
Bécancour	50.05	0.0605	0.0656	NEG	0	N	.	.	0.93
Bécancour	50.05	0.0605	0.0656	NEG	0	Y	.	.	0.91
Boucherville	50.42	0.7264	0.0101	NEG	0	N	.	.	1.03
Boucherville	50.42	0.7264	0.1012	MARG	2	Y	3.35E+00	3.80E-02	1.16
Contrecoeur	50.15	0.6997	0.0590	NEG	0	N	.	.	0.89
Contrecoeur	50.15	0.6997	0.0590	M/Erratic	2	Y	.	.	1.07
Donnacoona	50.06	0.3272	0.0159	NEG	0	N	.	.	0.94
Donnacoona	50.06	0.3272	0.0159	MARG	1	Y	6.50E-01	2.65E-01	1.03
Jonquière	41.19	0.6129	0.0526	POS	5	N	9.76E-01	1.50E-02	1.10
Jonquière	41.19	0.6129	0.0526	POS	6	Y	2.13E+01	1.69E-02	1.64
La Prairie	50.07	0.7459	0.0847	NEG	0	N	.	.	0.94
La Prairie	50.07	0.7459	0.0847	NEG	0	Y	.	.	0.87
La Baie	50.00	0.2997	0.0218	M/Erratic	2	N	.	.	1.03
La Baie	50.00	0.2997	0.0218	POS	4	Y	7.14E-01	1.05E-01	1.16
Longueuil	50.00	0.6594	0.0753	NEG	0	N	.	.	0.88
Longueuil	50.00	0.6594	0.0753	M/Erratic	1	Y	.	.	1.03
La Tuque 1-91	50.21	0.5351	0.0404	NEG	0	N	.	.	0.98
La Tuque 1-91	50.21	0.5351	0.0404	NEG	0	Y	.	.	0.80
La Tuque 2-92	45.60	0.5794	0.0387	NEG	0	N	.	.	0.92
La Tuque 2-92	45.60	0.5794	0.0387	NEG	0	Y	.	.	0.78
La Tuque 2-91	49.96	0.6953	0.0701	NEG	0	N	.	.	0.96
La Tuque 2-91	49.96	0.6953	0.0701	NEG	0	Y	.	.	0.93
La Tuque 1-92	56.71	0.6542	0.0791	NEG	0	N	.	.	1.02
La Tuque 1-92	56.71	0.6542	0.0791	NEG	0	Y	.	.	0.92
Montréal-East	50.49	0.3667	0.0272	POS	4	N	1.68E+00	7.30E-02	1.21

Montréal-East	50.49	0.3667	0.0272	POS	5	Y	1.79E+01	6.40E-03	1.44
Pt-aux-Trembles	50.00	0.3377	0.0140	NEG	0	N	.	.	0.87
Pt-aux-Trembles	50.00	0.3377	0.0140	NEG	0	Y	.	.	1.03
Tracy	50.12	0.6647	0.0404	NEG	0	N	.	.	0.85
Tracy	50.12	0.6647	0.0404	NEG	0	Y	.	.	0.88
Sorel	50.46	0.5371	0.0250	NEG	0	N	.	.	0.92
Sorel	50.46	0.5371	0.0250	NEG	0	Y	.	.	0.84
St. Romuald	50.00	0.2481	0.0138	POS	5	N	2.63E-01	.	1.11
St. Romuald	50.00	0.2481	0.0138	MARG	2	Y	2.28E-01	2.97E-01	1.18
Trois Rivières	50.00	0.4387	0.0343	NEG	0	N	.	.	1.00
Trois Rivières	50.00	0.4387	0.0343	MARG	2	Y	1.87E+00	6.70E-02	1.17
Varennnes	50.16	0.3129	0.0193	M/Erratic	1	N	.	.	1.05
Varennnes	50.16	0.3129	0.0193	Erratic	4	Y	.	.	1.16
Memphremagog	55.84	0.8120	0.1740	NEG	0	N	.	.	0.76
Memphremagog	55.84	0.8120	0.1740	NEG	0	Y	.	.	0.72
Spiked Sediment [†]	50.21	0.5351	0.0440	POS	6	Y	1.31E+06	.	2.10
TCL PAH Mix [*]	.	.	.	POS	6	Y	5.20E+06	.	3.00

[†]Bottom sediment from La Tuque site 1 spiked with 200 µL of Supelco TCL PAH Mix # 4-8905 (Supelco Canada Inc., Oakville, Ontario).

^{*}Supelco TCL PAH Mix alone.

4) Suspended particulate matter genotoxicity results used in Figures 3, 4 and 5 of Chapter 5.

Variables:

Extracted = Quantity of sediment extracted and subjected to GPC clean-up in grams dry wt.

Response = SOS Chromotest result.

Above = Number of concentrations that exceed upper confidence limit of control.

Activ. = S9 Activation status.

SRIP = SOS Response Inducing Potency in IF Units per g dry sediment per assay mL.

LOGC = Lowest Observable Genotoxic Concentration in g dry sediment.

MaxIF = Maximum Induction Factor Observed.

Org. = Organic matter content - Loss on ignition at 550 °C, fraction of dry sediment (From NAQUADAT 1991 and 1992 Data).

Site	Sample	Extracted	Response	Above	Activ.	SRIP	LOGC	MaxIF	Organic
Alma	SSed	0.111	POS	4	N	7.66E+01	1.13E-03	2.11	
Alma	SSed	0.111	POS	3	Y	3.15E+00	1.01E-02	1.33	
Bécancour	SSed	0.153	POS	6	N	2.68E+01	3.51E-03	1.75	

Bécancour	SSed	0.153	MARG	1	Y	2.60E+00	8.49E-02	1.16	
Boucherville	SSed	0.184	POS	6	N	1.34E+02	3.19E-03	1.60	18.9
Boucherville	SSed	0.184	Erratic	3	Y	.	.	1.08	18.9
Contrecoeur	SSed	0.254	POS	6	N	5.51E+01	1.72E-03	1.98	20.1
Contrecoeur	SSed	0.254	MARG	1	Y	7.57E+00	3.16E-02	1.39	20.1
Donnacoona	SSed	1.658	POS	6	N	3.53E+00	9.37E-03	1.36	
Donnacoona	SSed	1.658	M/Erratic	1	Y	.	.	1.05	
Jonquière	SSed	1.114	POS	6	N	1.71E+01	7.53E-03	2.16	
Jonquière	SSed	1.114	POS	6	Y	6.13E-01	1.36E-02	1.62	
La Tuque 1-91	SSed	0.273	POS	6	N	3.70E+00	.	1.70	
La Tuque 1-91	SSed	0.273	NEG	0	Y	.	.	1.11	
La Tuque 1-92	SSed	1.785	POS	5	N	2.10E+00	4.85E-02	3.40	
La Tuque 1-92	SSed	1.785	POS	4	Y	2.12E-01	1.89E-01	1.29	
La Tuque 2-91	SSed	0.213	POS	6	N	9.60E+00	5.47E-03	2.05	
La Tuque 2-91	SSed	0.213	POS	5	Y	2.83E+00	8.68E-03	1.29	
La Tuque 2-92	SSed	1.394	POS	6	N	4.00E+00	.	4.38	
La Tuque 2-92	SSed	1.394	POS	5	Y	6.54E-01	9.64E-02	1.71	
La Bale	SSed	1.700	NEG	0	N	.	.	1.03	
La Bale	SSed	1.700	NEG	0	Y	.	.		
La Prairie	SSed	0.159	POS	5	N	3.52E+01	4.04E-03	2.53	
La Prairie	SSed	0.159	MARG	2	Y	1.80E+00	4.47E-02	1.08	
Longueuil	SSed	0.308	POS	5	N	2.00E+01	1.90E-03	1.81	
Longueuil	SSed	0.308	POS	6	Y	1.16E+00	1.21E-02	1.31	
Beauharnois	SSed	0.105	POS	6	N	1.02E+02	1.89E-03	2.98	
Beauharnois	SSed	0.105	POS	5	Y	7.06E+00	7.52E-03	1.37	
Montréal-East	SSed	0.180	POS	6	N	4.16E+01	1.90E-06	5.00	14.6
Montréal-East	SSed	0.180	POS	6	Y	7.36E+00	2.28E-03	2.03	14.6
Pt.-aux-Trembles	SSed	0.168	POS	6	N	8.89E+00	.	2.48	
Pt.-aux-Trembles	SSed	0.168	NEG	0	Y	.	.	1.01	
Tracy	SSed	0.307	POS	4	N	1.32E+00	1.85E-02	1.28	21.5
Tracy	SSed	0.307	NEG	0	Y	.	.		21.5
Sorel	SSed	0.293	POS	5	N	5.04E+01	6.58E-03	1.41	16.0
Sorel	SSed	0.293	NEG	0	Y	.	.		16.0
St. Romuald	SSed	1.258	POS	6	N	4.16E+00	.	3.30	
St. Romuald	SSed	1.258	POS	6	Y	3.82E-01	1.42E-02	1.22	
Trois Rivières	SSed	0.153	POS	5	N	1.39E+01	1.76E-03	1.40	
Trois Rivières	SSed	0.153	MARG	2	Y	1.62E+00	4.89E-02	1.16	
Varennes	SSed	0.203	POS	4	N	1.81E+01	1.08E-02	3.20	7.2
Varennes	SSed	0.203	POS	5	Y	4.61E+00	8.00E-03	1.45	7.2

Memphremagog	SSed	0.032	POS	4	N	5.87E+02	9.68E-04	1.20
Memphremagog	SSed	0.032	MARG	2	Y	4.10E+01	3.24E-03	1.13
Spiked Sample†	SSed	.	POS	6	Y	.	.	2.23

†Suspended sediment from Lake Memphremagog spiked with 200 µL of Supelco TCL PAH Mix # 4-8905 (Supelco Canada Inc., Oakville, Ontario). Strong + response. SRIP not calculated because response far out of range.

5) Regional industrial effluent data used in Figures 4 and 8 of Chapter 5.

Variables:

SRIP values- All are weighted averages of direct acting genotoxic potency values.

Acid SRIP = Weighted average SRIP of aqueous acid extract in IF Units per mL.

Base SRIP = Weighted average SRIP of aqueous alkaline extract in IF Units per mL.

Solid SRIP = Weighted average SRIP of suspended solid extract in IF Units per g dry wt.

Load values- All are maximum, regional genotoxic loading values (Max - uses activation conditions that provide the largest potency values).

Acid Load = Regional genotoxic average SRIP of aqueous acidic substances in IF Units per day.

Base Load = Regional genotoxic average SRIP of aqueous alkaline substances in IF Units per day.

Solid Load = Regional genotoxic average SRIP of substances associated with suspended particulate matter in IF Units per day.

Region	Acid SRIP	Base SRIP	Solid SRIP	Acid Load	Base Load	Solid Load
Alma	3.46E-03	.	3.59E+03	4.58E+07	.	4.56E+07
Beauharnois	4.43E-03	1.88E-02	1.21E+03	1.31E+08	5.51E+08	2.58E+08
Bécancour	2.73E-02	.	3.20E+02	2.12E+08	.	2.92E+07
Boucherville	5.30E-02	1.01E-02	3.05E+00	1.74E+06	1.13E+07	2.79E+05
Contrecoeur	1.67E-02	9.30E-02	1.32E+03	7.58E+08	4.21E+09	1.38E+09
Donnacoona	1.48E-03	9.20E-04	.	4.31E+08	5.93E+07	.
Jonquière	3.13E-03	3.29E-02	2.19E+02	7.47E+08	7.85E+09	2.50E+09
La Baie	9.28E-03	4.46E-03	2.52E+00	5.74E+08	2.76E+08	2.21E+07
La Prairie	.	.	1.51E+01	.	.	1.08E+06
Longueuil	9.23E-02	.	5.95E+02	3.15E+08	.	1.10E+08
Montréal-East	3.05E-02	3.04E-02	1.08E+03	1.03E+09	1.18E+09	9.09E+08
Pt.-aux-Trembles	.	4.72E-02	.	1.25E+09	7.63E+10	.

Tracy	9.94E-02	.	1.90E+00	1.87E+10	.	4.41E+08
Sorel	6.92E-02	.	1.72E+01	5.40E+09	.	7.80E+07
St Romuald	9.96E-03	.	.	9.42E+07	3.46E+06	.
Trois Rivières	1.52E-01	5.48E-03	6.91E+02	1.18E+10	4.22E+08	1.92E+08
Varenes	3.29E-02	9.99E-03	1.72E+02	2.11E+09	6.32E+08	6.56E+08

APPENDIX 8

Data Used to Generate Tables and Figures in Chapter 6

A) Summary of surface water genotoxicity data originally published by Langevin et al. (1992).

Site	Total Susp. Particulates (mg per L) [‡]	MDGC Whole Water (mL) [*]	Conc. of BaP Equivalents (ng per L) [§]	Discharge (L per Day) [†]
Ottawa River				
L. Two Mountains	4.5	251.5	301.0	9.90 x 10 ¹⁰
St. Lawrence River				
Lake St. Francis	2.5	185.0	409.2	6.01 x 10 ¹¹
Montréal Area	5.4	148.4	510.1	6.51 x 10 ¹¹

[‡]1985-1990 mean values. N = 23 to 358. Data from Rondeau, B. 1993. Qualité des Eaux du Fleuve Saint-Laurent 1985-1990. Tronçon Cornwall-Québec. Direction Écotoxicologie et Écosystèmes, Centre Saint-Laurent, Conservation et Protection, Environnement Canada, Montréal, Québec.

^{*}Geometric mean values expressed as equivalent mL per assay mL.

[§]Calculated using the measured mean MDGC of Benzo(a)pyrene on the SOS Chromotest (0.0757 µg per assay mL)

[†]Mean summer discharge for 1988. Data from Environment Canada (1992).

B) Summary of Montreal area loading values data used to generate Figure 1.

Montréal regional contributions to surface water genotoxicity-

Loadings come from--

- 1) MUC (Montreal Urban Community) municipal wastewater treatment system.
- 2) Regional industrial loadings from 12 industries (examined in Chapters 3 and 4).
(3 discharged into MUC system + 9 that discharge directly into the St. Lawrence river).
- 3) Contributions from inhabitants not connected to MUC system.

#1 above further broken down into --

- 1) Large Industries (3 examined in Chapter 3) - 2.0% of Total Loading
- 2) Small (uncharacterized) industries - 6.8%.
(Calculated as described in Figure 1 legend).
- 3) Domestic (Non-Industrial) - 91.2%

The 91.2% of 31.1 kg of BaP equiv. per day from MUC wastewater collection system
= 28.4 kg BaP Equiv. per day + 1.4×10^6 people
⇒ 20.3 mg BaP per person per day.

Summary of Montréal Regional Contributions

Source	Total Loading (g BaP per day)	% of Total Loading
Industrial Sources-		
1) Large Industries (MUC) [†]	622.4	1.2
2) Large Industries (direct) [†]	3898.1	7.6
3) Unknown Small Industries [*]	2117.4	4.1

Industrial Total	6637.9	12.9
Domestic Sources-		
1) MUC Loading [§]	28367.2	55.3
2) Non-MUC Loading [†]	16291.5	31.8
Domestic Total	44658.7	87.1
Total Loading	51296.6	

[†]Examined in Chapters 3 and 4.

^{*}Calculated as described in Figure 1 legend of Chapter 6.

[§]Total load of MUC wastewater treatment plant - industrial loading above.

[†]Calculated from *per capita* emission rate and population of communities along the south shore of the St. Lawrence river (8×10^5 people).

B) Data used to generate Great Lakes results presented in Table 1, Chapter 6.

Notes on Variables-

Domestic Load (ng per year) = $20.3 \text{ mg/person/day} \times \text{Pop} \times 10^6 \text{ ng/mg} \times 365 \text{ days/year}$.

Discharge of lake (L/yr) = volume (L)/residence time (yrs).

Fraction adsorbed = calculated from K_d -Geno of domestic wastes ($\approx 10^4$) and concentration of suspended particulate matter.

Settling velocity = sedimentation rate ($\text{g m}^{-2} \text{ yr}^{-1}$)/concentration of suspended sediment (g m^{-3}).

Loss to sedimentation per yr = fraction adsorbed * (settling rate (m yr^{-1})/Mean depth(m)).

Adjusted Outflow in L yr^{-1} = (Outflow as volume/residence time) + [(loss to sedimentation (yr^{-1}) * volume (L)] + [(Loss to decay (yr^{-1}) * Volume (L)).

Outflow Concentration = Sum of genotoxic inputs (ng yr^{-1}) / corrected outflow rate (L yr^{-1}).

Daily Genotoxic Export = (Outflow conc (ng L^{-1}) * Discharge (L yr^{-1}))/365.

Total Losses as % = [((outflow Concentration (ng L^{-1}) if No Losses ie. W/Q)-(actual outflow concentration (ng L^{-1}))/(W/Q))*100.

% Loss to Sedimentation = [(Sedimentation Loss (yr^{-1}) * Lake Volume (L))/Corrected Outflow rate (L yr^{-1}))*100.

% Loss to Decay = [(Decay loss (yr^{-1}) * Lake Volume (L))/Corrected Outflow Rate (L yr^{-1}))*100.

W/Q = Total Load / discharge. Provides concentration under conditions of 0% loss.

Lake	Pop (x 10 ⁶)	Domestic Load (ng yr ⁻¹)	Lake Volume (L)	Res. Time (yrs)	Discharge (L yr ⁻¹)	Fraction Adsorbed	Sed Rate (g m ⁻² yr ⁻¹)	Tot Susp Solids (g m ⁻³)	Settling Vel (m yr ⁻¹)
Superior	0.74	5.47E+15	1.21E+16	191	6.34E+13	1.37E-02	138	1.39	99.28
Michigan	13.97	1.04E+17	4.92E+15	99	4.97E+13	1.38E-02	151	1.40	107.86
Huron	2.37	1.76E+16	3.54E+15	22	1.61E+14	3.30E-02	284	3.41	83.28
Erie	12.97	9.61E+16	4.84E+14	2.6	1.86E+14	19.07E-01	580	23.56	24.62
Ontario	6.64	4.92E+16	1.64E+15	6	2.73E+14	7.34E-02	445	7.92	56.19
St. Lawrence to Cornwall	0.42	3.11E+15			2.31E+14†				

Lake	Mean Depth (m)	Loss to Sed (yr ⁻¹)	Adjusted Outflow Rate (L yr ⁻¹)	Outflow Conc (ng yr ⁻¹)	Geno. Discharge (ng BaP Eq. Day ⁻¹)	Total Loss (%)	W/Q	Sed Loss (%)	Decay Loss (%)
Superior	147	9.26E-03	1.74E+15	3.15	5.47E+11	96.4	86.38	6.4	89.9
Michigan	85	1.75E-02	7.71E+14	134.34	1.83E+13	93.6	2082.97	11.2	82.4
Huron	59	4.65E-02	7.82E+14	31.25	1.38E+13	79.4	151.96	21.1	58.4
Erie	19	2.47E-01	3.68E+14	274.66	1.40E+14	49.4	543.21	32.5	17.0
Ontario	86	4.79E-02	5.63E+14	178.06	1.33E+14	51.5	367.11	14.0	37.5
St. Lawrence to Cornwall				223.71	8.53E+12	0.0	223.71		

Predicted Conc
@ Cornwall

224 1.42E+14
ng per L ng BaP equiv per Day

†30 yr mean discharge @ Cornwall

3) *Salmonella*/Mammalian Microsome assay data used to generate Figure 3, Chapter 6.

Variables:

Population = Inhabitants of drainage basin above sampling site (as described in Chapter 6).

Discharge = River discharge adjusted for sampling season wherever possible.

Strain/Activ. = *Salmonella typhimurium* strain and S9 activation status.

Mut. Potency = Ames Mutagenic potency in Revertents per mL equivalent of surface water per assay mL (ie. mL of top agar). Values are whole water values adjusted for filtration losses.

River	Location	Population	Sampling Date	Discharge (L Day ⁻¹)	Strain/Activ.	Mut. Potency
Po	Ferrara	5.23E+06	Sept. 88	8.89E+10	TA98 NoS9	2.74E-01
Po	Ferrara	5.23E+06	Nov. 88	1.19E+11	TA98 NoS9	5.04E-01
Po	Porto Tolle	5.23E+06	1991-1992	6.91E+10	TA98 NoS9	1.04E-01
Rhine	Lobith	3.00E+07	Apr. 79	2.40E+11	TA98 NoS9	5.68E-01
Rhine	Lobith	3.00E+07	Jun. 79	2.17E+11	TA98 NoS9	7.65E-01
Rhine	Lobith	3.00E+07	Oct. 79	1.43E+11	TA98 NoS9	2.39E+00
Rhine	Lobith	3.00E+07	Feb. 80	2.43E+11	TA98 NoS9	4.49E-01
Rhine	Lobith	3.00E+07	Spring	2.26E+11	TA98 NoS9	5.55E-01
Rhine	Lobith	3.00E+07	Autumn	1.54E+11	TA98 NoS9	1.09E+00
Rhine	Gorinchem	3.05E+07	Apr. 79	1.41E+11	TA98 NoS9	7.56E-01
Rhine	Gorinchem	3.05E+07	Jun. 79	1.58E+11	TA98 NoS9	1.41E+00
Rhine	Gorinchem	3.05E+07	Oct. 79	8.81E+10	TA98 NoS9	3.81E+00
Rhine	Gorinchem	3.05E+07	Feb. 80	3.87E+11	TA98 NoS9	6.07E-01
Rhine	Gorinchem	3.05E+07	79 to 80	1.94E+11	TA98 NoS9	4.05E-01
Rhine	Gorinchem	3.05E+07	79 to 80	1.94E+11	TA98 NoS9	5.16E-01
Rhine	Werkendam	3.05E+07	Autumn	1.81E+11	TA98 NoS9	1.04E+00
Rhine	Gorinchem	3.05E+07	1978-79	2.40E+11	TA98 NoS9	4.64E-01
Yodo	Kyoto	1.44E+06	1981-82	2.65E+10	TA98 NoS9	2.48E-01
Llobregat	Barcelona	1.00E+06	Feb-July 86	4.00E+09	TA98 NoS9	2.26E+00
Vaal	Sasolburg	1.26E+06	1980?	5.90E+09	TA98 NoS9	2.02E+00
Adige	Anguillara	6.52E+05	1991-92	2.00E+10	TA98 NoS9	8.13E-02
Po	Ferrara	5.23E+06	Sept. 88	8.89E+10	TA98 +S9	4.14E-01
Po	Ferrara	5.23E+06	Nov. 88	1.19E+11	TA98 +S9	1.78E-01
Po	Ferrara	5.23E+06	Jan. 89	6.18E+10	TA98 +S9	3.77E-01
Po	Ferrara	5.23E+06	Feb. 89	1.89E+11	TA98 +S9	5.67E-01
Po	Ferrara	5.23E+06	Apr. 89	1.99E+11	TA98 +S9	3.84E-01
Po	Ferrara	5.23E+06	May 89	1.98E+11	TA98 +S9	6.88E-01
Po	Ferrara	5.23E+06	Jun. 89	9.68E+10	TA98 +S9	4.65E-01
Po	Ferrara	5.23E+06	Jul. 89	1.20E+11	TA98 +S9	4.97E-01
Po	Ferrara	5.23E+06	Aug. 89	6.54E+10	TA98 +S9	3.86E-01
Po	Ferrara	5.23E+06	Sept. 89	7.21E+10	TA98 +S9	5.19E-01

Po	Porto Tolle	5.23E+06	1991-1992	6.91E+10	TA98 +S9	2.47E-01
Po	Porto Tolle	5.23E+06	1991-1992	6.91E+10	TA98 +S9	2.97E-01
Po	Porto Tolle	5.23E+06	1991-1992	6.91E+10	TA98 +S9	1.28E-01
Po	Porto Tolle	5.23E+06	1991-1992	6.91E+10	TA98 +S9	1.36E-01
Rhine	Lobith	3.00E+07	Apr. 79	2.40E+11	TA98 +S9	2.70E+00
Rhine	Lobith	3.00E+07	Jun. 79	2.17E+11	TA98 +S9	1.79E+00
Rhine	Lobith	3.00E+07	Oct. 79	1.43E+11	TA98 +S9	7.95E+00
Rhine	Lobith	3.00E+07	Feb. 80	2.43E+11	TA98 +S9	1.27E+00
Rhine	Gorinchem	3.05E+07	Apr. 79	1.41E+11	TA98 +S9	3.93E+00
Rhine	Gorinchem	3.05E+07	Jun. 79	1.58E+11	TA98 +S9	2.06E+00
Rhine	Gorinchem	3.05E+07	Oct. 79	8.81E+10	TA98 +S9	8.76E+00
Rhine	Gorinchem	3.05E+07	Feb. 80	3.87E+11	TA98 +S9	1.62E+00
Rhine	Gorinchem	3.05E+07	79 to 80	1.94E+11	TA98 +S9	9.15E-01
Rhine	Gorinchem	3.05E+07	79 to 80	1.94E+11	TA98 +S9	1.13E+00
Rhine	Lobith	3.00E+07	Spring	2.26E+11	TA98 +S9	1.93E+00
Rhine	Lobith	3.00E+07	Autumn	1.54E+11	TA98 +S9	4.51E+00
Rhine	Werkendam	3.05E+07	Autumn	1.81E+11	TA98 +S9	1.40E+00
Rhine	Lobith	3.00E+07	1978-79	2.18E+11	TA98 +S9	2.17E+00
Rhine	Gorinchem	3.05E+07	1978-79	2.40E+11	TA98 +S9	2.28E+00
Yodo	Kyoto	1.44E+06	1981-82	2.65E+10	TA98 +S9	1.69E+00
Llobregat	Barcelona	1.00E+06	Feb-July 86	4.00E+09	TA98 +S9	3.88E+00
Vaal	Sasolburg	1.26E+06	1980?	5.90E+09	TA98 +S9	3.84E+00
Adige	Anguillara	6.52E+05	1991-1992	2.00E+10	TA98 +S9	2.14E-01

APPENDIX 9

Data Used to Generate Tables and Figures in Chapter 7

A) Figure 1 Data-- concentration response for 4 *Mya arenaria* extracts.

Variables:

Concentration = equivalent g wet tissue per assay mL.

MeanIF = Mean SOS Induction factor.

Site	Sample	Concentration	Mean IF	Std. Error
Baie Éternité	1 of 1	0.768	1.07	0.0035
Baie Éternité	1 of 1	1.537	1.32	0.0487
Baie Éternité	1 of 1	3.074	1.56	0.0243
Baie Éternité	1 of 1	6.148	2.55	0.1289
Baie Éternité	1 of 1	12.295	3.16	0.0138
Baie Éternité	1 of 1	24.590	3.68	0.5500
Grosse Roche	1 of 1	0.836	1.38	0.2181
Grosse Roche	1 of 1	1.671	1.96	0.0037
Grosse Roche	1 of 1	3.342	2.57	0.0837
Grosse Roche	1 of 1	6.684	3.09	0.0805
Grosse Roche	1 of 1	13.368	2.59	0.1047
Grosse Roche	1 of 1	26.735	1.82	0.2053
St. Étienne C.	2 of 2	0.558	1.07	0.0970
St. Étienne C.	2 of 2	1.115	1.36	0.0454
St. Étienne C.	2 of 2	2.231	1.83	0.0347
St. Étienne C.	2 of 2	4.461	2.41	0.0341
St. Étienne C.	2 of 2	8.923	3.10	0.3573
St. Étienne C.	2 of 2	17.845	4.60	0.1713
St. Jean	1 of 2	0.811	1.12	0.0235
St. Jean	1 of 2	1.622	1.20	0.0617
St. Jean	1 of 2	3.244	1.50	0.0098
St. Jean	1 of 2	6.488	1.97	0.1418
St. Jean	1 of 2	12.975	2.17	0.0489
St. Jean	1 of 2	25.950	3.00	0.2685

2) *Mya arenaria* and *Mytilus edulis* genotoxicity results used in Figures 2 and 3 of Chapter 7.

Variables:

Extracted = Quantity of tissue extracted in grams wet wt.

Response = SOS Chromotest result.

Above = Number of concentrations that exceed upper confidence limit of control.

Activ. = S9 Activation status.

SRIP-wet = SOS Response Inducing Potency in IF Units per g wet tissue per assay mL.

SRIP-lip = SOS Response Inducing Potency in IF Units per mg lipid per assay mL.

LOGC-wet = Lowest Observable Genotoxic Concentration in g wet tissue.

LOGC-lip = Lowest Observable Genotoxic Concentration in mg lipid.

MaxIF = Maximum Induction Factor Observed.

Site	Sample	Extract	Resp.	Above	Activ.	SRIP-wet	SRIP-Lip	LOGC-Wet	LOGC-Lip	Lipid	MaxIF
Baie Éternité	<i>Mya</i>	60.0	POS	6	N	2.10E-01	3.28E-02	7.74E-01	4.95	0.64	3.70
Baie Éternité	<i>Mya</i>	60.0	POS	5	Y	2.56E-02	4.00E-03	.	.	0.64	1.64
Baie Ste-Cath	<i>Mytilus</i>	25.3	POS	6	N	2.04E-01	5.37E-02	6.07E-01	2.31	0.38	2.73
Baie Ste-Cath	<i>Mytilus</i>	25.3	MARG	1	Y	7.10E-02	1.87E-02	8.91E+00	33.86	0.38	1.28
A. à la Barque	<i>Mya</i> (1 of 2)	53.7	MARG	1	N	.	.	.	.	0.38	1.11
A. à la Barque	<i>Mya</i> (1 of 2)	53.7	NEG	0	Y	.	.	.	.	0.38	.
A. à la Barque	<i>Mya</i> (2 of 2)	45.8	POS	6	N	5.61E-01	8.51E-02	4.47E-01	2.95	0.66	2.03
A. à la Barque	<i>Mya</i> (2 of 2)	45.8	POS	3	Y	7.55E-02	1.14E-02	1.54E+00	10.16	0.66	1.19
A. à la Barque	<i>Mytilus</i>	16.8	POS	3	N	1.59E-01	2.89E-02	1.14E+00	6.27	0.55	2.16
A. à la Barque	<i>Mytilus</i>	16.8	POS	3	Y	7.69E-02	1.40E-02	4.75E+00	26.10	0.55	1.30
Moulin à Baude	<i>Mya</i> (1 of 2)	53.7	NEG	0	N	.	.	.	.	0.38	.
Moulin à Baude	<i>Mya</i> (1 of 2)	53.7	NEG	0	Y	.	.	.	.	0.38	.
Moulin à Baude	<i>Mya</i> (2 of 2)	53.9	POS	4	N	7.21E-02	6.55E-02	1.18E+00	1.30	0.11	1.28
Moulin à Baude	<i>Mya</i> (2 of 2)	53.9	MARG	2	Y	1.18E-02	1.07E-02	9.82E+00	10.81	0.11	1.19
Moulin à Baude	<i>Mytilus</i>	56.1	POS	6	N	2.92E-01	5.21E-02	6.82E-01	3.82	0.56	1.69
Moulin à Baude	<i>Mytilus</i>	56.1	POS	3	Y	2.54E-02	4.53E-03	3.89E+00	21.78	0.56	1.11
Grosse Roche	<i>Mya</i>	54.5	POS	6	N	6.00E-01	2.73E-01	1.52E+00	0.60	0.22	3.10
Grosse Roche	<i>Mya</i>	54.5	POS	6	Y	4.88E-02	8.13E-01	5.40E+00	11.88	0.22	1.62
Petit Saguenay	<i>Mya</i>	59.2	POS	6	N	3.49E-01	8.10E-02	1.55E+01	66.56	0.43	2.18
Petit Saguenay	<i>Mya</i>	59.2	POS	6	Y	9.17E-02	2.13E-02	.	.	0.43	2.31
St. Jean	<i>Mya</i> (1 of 2)	52.9	POS	6	N	1.52E-01	6.33E-02	3.27E-01	0.79	0.24	3.00
St. Jean	<i>Mya</i> (1 of 2)	52.9	POS	4	Y	9.60E-03	4.00E-03	3.59E+00	8.62	0.24	1.53

St. Jean	Mya (2 of 2)	36.2	POS	6	N	3.84E-01	1.83E-01	5.78E-02	0.12	0.21	2.64
St. Jean	Mya (2 of 2)	36.2	POS	3	Y	8.28E-02	3.94E-02	3.97E+00	8.33	0.21	2.41
St. Louis	Mya (1 of 2)	58.5	POS	6	N	3.53E-02	4.80E-03	1.74E+00	12.91	0.74	1.54
St. Louis	Mya (1 of 2)	58.5	POS	6	Y	4.38E-02	5.92E-03	8.34E-02	0.62	0.74	1.13
St. Louis	Mya (2 of 2)	58.7	POS	6	N	1.13E+00	1.88E-01	.	.	0.6	2.16
St. Louis	Mya (2 of 2)	58.7	POS	3	Y	6.94E-02	1.16E-02	2.45E+00	14.68	0.6	1.61
Ste. Étienne C.	Mya (1 of 2)	59.8	POS	6	N	3.29E-01	3.78E-02	3.63E-01	3.13	0.87	3.00
Ste. Étienne C.	Mya (1 of 2)	59.8	POS	3	Y	5.40E-02	6.21E-03	1.16E+00	10.13	0.87	1.35
Ste. Étienne C.	Mya (2 of 2)	56.5	POS	6	N	1.77E-01	3.22E-02	1.18E-01	0.65	0.55	4.60
Ste. Étienne C.	Mya (2 of 2)	56.5	POS	5	Y	8.95E-02	1.63E-02	2.00E+00	11.02	0.55	2.56
Ste. Étienne E.	Mya (1 of 2)	59.0	POS	6	N	1.54E-01	1.92E-02	1.83E+00	14.64	0.8	1.72
Ste. Étienne E.	Mya (1 of 2)	59.0	MARG	2	Y	5.92E-02	7.40E-03	4.46E+00	35.68	0.8	1.47
Ste. Étienne E.	Mya (2 of 2)	52.6	POS	3	N	2.45E-01	7.90E-02	1.08E+00	3.35	0.31	1.53
Ste. Étienne E.	Mya (2 of 2)	52.6	MARG	2	Y	1.11E-02	1.96E-03	4.12E+00	23.09	0.31	1.06
Ste. Étienne W.	Mya (1 of 2)	58.6	POS	4	N	2.30E-02	3.43E-03	2.27E+00	15.23	0.67	1.21
Ste. Étienne W.	Mya (1 of 2)	58.6	POS	3	Y	1.19E-02	1.77E-03	2.90E+00	19.41	0.67	1.22
Ste. Étienne W.	Mya (2 of 2)	54.7	POS	6	N	1.83E-01	4.25E-02	2.00E+00	8.61	0.43	5.60
Ste. Étienne W.	Mya (2 of 2)	54.7	POS	6	Y	9.13E-02	2.12E-02	9.37E-02	4.03	0.43	2.06
Clam Spike†	.	24.6	POS	6	Y	.	.	.	.	0.34	2.43

†Unionid bivalve from Lake Memphremagog spiked with 200 µL of Supelco TCL PAH Mix # 4-8905 (Supelco Canada Inc., Oakville, Ontario).
Strong + response. SRIP not calculated because response far out of range.

3) *Mya arenaria* and *Mytilus edulis* PAH results used in Figures 3 and Table 4 of Chapter 7.

Variables:

Total = Sum concentration of 21 measured PAHs listed in Table 2, Chapter 7 in ppb (ng per g wet tissue).

All remaining variables = concentration of PAH in ppb (ng per g wet tissue). Abbreviations are the same as those used in Tables 2 and 4 of Chapter 7.

Site	Sample	Total	PHEN	ANTH	FLUOR	PYR	B(c)P	B(a)A	CHRY
B. Éternité	Mya	57.8	0.00	2.99	12.95	9.96	1.00	2.99	16.93
Ste-Catherine	Mytilus	16.8	0.00	1.98	3.95	2.96	0.00	0.00	3.95
A. à la Barque	Mya (1/2)	18.0	0.00	0.00	2.99	2.00	0.00	2.00	4.99
A. à la Barque	Mya (2/2)	14.9	0.00	1.87	0.00	3.73	0.00	0.00	5.60
A. à la Barque	Mytilus	23.8	0.00	1.98	4.96	3.97	0.00	0.99	5.95
B. Moulin à Baude	Mya (1/2)	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00

B. Moulin à Baude	Mya (2/2)	8.8	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B. Moulin à Baude	Mytilus	1.0	0.00	0.00	0.00	0.00	0.00	0.00	1.00
Grosse Roche	Mya	1399.6	0.00	0.00	3.86	2.90	8.69	5.79	6.76
Petit-Saguenay	Mya	53.6	0.00	1.98	10.91	7.94	0.99	3.97	15.87
Anse St. Jean	Mya (2/2)	37.8	1.99	0.00	6.96	5.96	0.00	1.99	9.94
Anse St. Jean	Mya (1/2)	36.8	0.00	1.00	5.97	4.98	0.00	1.99	9.95
Anse St. Louis	Mya (2/2)	41.8	1.94	0.00	8.75	6.80	0.00	0.97	14.58
Anse St. Louis	Mya (1/2)	66.3	0.00	1.95	13.65	9.75	1.95	2.92	17.54
Ste-Étienne-C	Mya (2/2)	42.6	0.00	0.99	7.93	5.95	0.00	1.98	14.87
Ste-Étienne-C	Mya (1/2)	38.5	0.00	1.98	8.89	5.93	0.00	1.98	9.88
Ste-Étienne-E	Mya (2/2)	32.8	0.00	0.97	4.83	3.86	0.00	1.93	8.69
Ste-Étienne-E	Mya (1/2)	41.1	1.96	0.00	8.81	5.88	0.00	1.96	11.75
Ste-Étienne-W	Mya (2/2)	27.8	0.00	1.99	5.96	3.98	0.00	1.99	6.96
Ste-Étienne-W	Mya (1/2)	40.0	0.00	0.00	3.00	4.00	1.00	2.00	15.98

Site	Sample	B(bjk)F	DMBA	B(a)P	3-MC	I(123cd)P	DB(ah)A	B(ghi)P	DB(al)P
B. Éternité	Mya	6.97	0.00	1.99	0.00	0.00	0.00	1.99	0.00
Ste-Catherine	Mytilus	2.96	0.00	0.00	0.00	0.00	0.00	0.00	0.99
A. à la Barque	Mya (1/2)	3.99	0.00	1.00	0.00	0.00	0.00	1.00	0.00
A. à la Barque	Mya (2/2)	2.80	0.00	0.93	0.00	0.00	0.00	0.00	0.00
A. à la Barque	Mytilus	4.96	0.00	0.00	0.00	0.00	0.00	0.99	0.00
B. Moulin à Baude	Mya (1/2)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B. Moulin à Baude	Mya (2/2)	1.95	0.00	0.98	0.00	1.95	0.00	3.91	0.00
B. Moulin à Baude	Mytilus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Grosse Roche	Mya	4.83	1158.30	4.83	32.82	164.09	4.83	1.93	0.00
Petit-Saguenay	Mya	7.94	0.00	1.98	0.00	0.00	0.00	1.98	0.00
Anse St. Jean	Mya (2/2)	7.95	0.00	2.98	0.00	0.00	0.00	0.00	0.00
Anse St. Jean	Mya (1/2)	5.97	0.00	1.99	0.00	1.99	0.00	2.99	0.00
Anse St. Louis	Mya (2/2)	8.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Anse St. Louis	Mya (1/2)	12.67	0.00	1.95	0.00	0.00	0.00	3.90	0.00
Ste-Étienne-C	Mya (2/2)	6.94	0.00	1.98	0.00	0.00	0.00	1.98	0.00
Ste-Étienne-C	Mya (1/2)	4.94	0.00	1.98	0.00	0.00	0.00	2.96	0.00
Ste-Étienne-E	Mya (2/2)	6.76	0.00	2.90	0.00	0.00	0.00	2.90	0.00
Ste-Étienne-E	Mya (1/2)	8.81	0.00	1.96	0.00	0.00	0.00	0.00	0.00
Ste-Étienne-W	Mya (2/2)	3.98	0.00	0.99	0.00	0.00	0.00	1.99	0.00
Ste-Étienne-W	Mya (1/2)	8.99	0.00	3.00	0.00	0.00	0.00	2.00	0.00

APPENDIX 10

Data Used to Generate Figures and Tables in Chapter 8

1) Genotoxicity of fish tissue extracts.

Variables:

Sample = Type of fish collected (see footnote).

TL = Total length in cm.

Resp. = SOS Chromotest result.

Above = Number of concentrations that exceeded control.

Activ. = S9 Activation status.

Lipid = Lipid content (% wet tissue wt.).

SRIP-Wet = Genotoxic potency in IF Units per equivalent g of wet tissue.

LOGC-Wet = Lowest observable genotoxic concentration in equivalent g wet tissue per assay mL.

MaxIF = Maximum Induction Factor observed

Site	Sample	TL (cm)	Resp.	Above	Activ.	Lipid	SRIP-Wet	LOGC-Wet	MaxIF
Alma	Pike	69.0	MARG	1	N	1.20	1.83E-01	3.86E+00	1.40
Alma	Pike	69.0	MARG	1	Y	1.20	1.85E-02	7.90E+00	1.12
Beauharnois	Perch	20.0	MARG	2	N	0.53	1.93E-02	3.25E+00	1.13
Beauharnois	Perch	11.5	MARG	2	N	3.33	3.60E+00	9.60E-02	1.83
Beauharnois	Perch	21.0	MARG	1	N	.	.	.	.
Beauharnois	Perch	20.0	POS	4	N	1.80	3.08E-02	4.66E+00	1.15
Beauharnois	Perch	12.0	POS	6	N	2.30	1.98E+00	6.90E-02	1.97
Beauharnois	Perch	20.0	POS	6	N	1.80	1.09E+00	.	1.81
Beauharnois	Perch	20.0	POS	4	N	0.53	2.79E-02	1.42E+00	1.23
Beauharnois	Perch	12.0	POS	3	Y	2.30	1.38E-01	8.85E-01	1.75
Beauharnois	Perch	11.5	MARG	1	Y	3.33	8.26E-01	4.10E-01	1.76
Beauport	Perch	25.3	MARG	1	N	1.30	3.50E-03	4.62E+00	.
Beauport	Perch	21.4	POS	3	N	1.80	1.17E-01	4.64E-01	.
Beauport	Walleye	36.5	POS	5	N	1.00	1.56E-02	2.86E+00	.
Beauport	Walleye	26.6	MARG	2	N	1.50	.	.	.
Beauport	Walleye	36.4	POS	5	N	1.00	4.60E-03	2.25E+00	.
Beauport	Walleye	27.6	POS	3	N	1.36	7.05E-02	1.44E+00	.
Beauport	Walleye	36.4	MARG	1	Y	1.00	2.73E-02	9.20E+00	.
Beaupré	Walleye	42.7	MARG	2	N	2.50	.	.	.

Beaupré	Walleye	35.5	MARG	1	N	1.60	.	.	.
Beaupré	Walleye	37.1	MARG	1	N	0.47	.	.	.
Bécancour	Perch	13.0	POS	3	N	2.30	6.75E-02	2.76E-01	1.09
Bécancour	Perch	13.0	MARG	2	N	2.50	6.04E+00	3.17E-02	1.15
Bécancour	Pike	55.6	MARG	2	N	1.20	1.25E+00	1.74E-01	1.28
Bécancour	Pike	57.5	POS	5	N	0.11	1.11E-01	3.95E-01	.
Bécancour	Pike	52.3	POS	5	N	1.10	2.52E-02	1.44E+00	1.28
Bécancour	Pike	57.5	POS	3	Y	0.11	2.25E-02	3.87E+00	1.25
Boucherville	Perch	13.9	MARG	2	N	3.68	2.95E+00	6.16E-02	1.17
Boucherville	Perch	13.5	POS	5	N	4.50	9.46E+00	4.82E-02	1.72
Boucherville	Perch	26.0	MARG	1	N	2.00	6.16E-01	2.57E-01	1.14
Boucherville	Perch	14.5	MARG	1	N	4.32	3.31E-01	5.53E-01	1.10
Boucherville	Perch	26.5	POS	5	N	0.59	4.89E-01	1.38E-01	1.22
Boucherville	Perch	13.5	POS	3	N	4.50	9.78E+00	3.22E-02	1.53
Boucherville	Perch	13.5	MARG	1	Y	4.50	2.57E-01	6.61E-01	1.26
Contrecoeur	Perch	24.0	POS	6	N	1.60	3.93E-01	5.56E-01	2.40
Contrecoeur	Perch	14.0	POS	3	N	3.20	2.27E-01	4.81E-01	1.81
Contrecoeur	Perch	23.5	POS	6	N	2.80	2.03E-01	4.46E-02	1.75
Contrecoeur	Perch	16.0	POS	6	N	3.90	3.87E-01	1.53E-01	1.95
Contrecoeur	Perch	13.0	POS	6	N	2.60	3.77E-01	5.19E-01	2.19
Contrecoeur	Perch	17.6	MARG	1	N	4.30	1.76E+00	1.11E-01	1.14
Contrecoeur	Perch	13.0	POS	6	N	2.60	4.47E-01	.	2.66
Contrecoeur	Perch	13.0	MARG	2	Y	2.60	2.64E-01	1.07E+00	1.60
Contrecoeur	Perch	13.0	MARG	1	Y	2.60	1.46E-01	1.52E+00	1.33
Contrecoeur	Perch	14.0	MARG	1	Y	3.20	1.37E-01	2.52E+00	1.17
Contrecoeur	Perch	16.0	MARG	2	Y	3.63	2.49E-01	9.78E-01	1.40
Contrecoeur	Perch	24.0	MARG	2	Y	1.60	1.08E-01	2.96E+00	1.26
Contrecoeur	Perch	23.5	MARG	1	Y	2.80	8.74E-02	2.55E+00	1.10
Contrecoeur	Pike	34.6	POS	5	N	0.70	1.28E-01	1.21E+00	1.90
Contrecoeur	Pike	38.8	POS	6	N	0.76	1.18E-01	.	2.52
Contrecoeur	Pike	34.6	MARG	1	Y	0.70	2.85E-02	1.41E+01	1.12
Contrecoeur	Pike	38.8	MARG	2	Y	0.76	8.40E-02	4.51E+00	1.16
Donnacona	Walleye	39.0	POS	4	N	1.40	1.61E-02	4.45E+00	.
Donnacona	Walleye	36.3	POS	6	N	0.93	4.45E-01	2.60E-01	1.18
Donnacona	Walleye	37.4	POS	5	N	0.89	2.35E-02	3.70E-01	.
Jonquière	Trout	21.8	POS	4	N	2.40	9.59E-02	5.93E-01	.
Jonquière	Trout	19.0	POS	3	N	2.00	7.14E-02	7.85E-01	1.36
Jonquière	Trout	23.5	MARG	2	N	1.60	6.65E-02	2.26E+00	1.11
La Bale	Herring	19.5	POS	4	N	6.90	4.24E+00	2.05E-02	1.38

La Baie	Herring	22.0	POS	5	N	10.60	1.09E+01	9.97E-03	1.36
La Baie	Sebastes	26.5	POS	3	N	6.40	2.75E+00	7.06E-02	1.14
La Baie	Sebastes	23.6	MARG	2	N	6.60	2.04E+00	1.38E-01	1.60
La Baie	Smelt	15.0	POS	3	N	2.30	2.42E+00	7.93E-02	1.30
La Prairie	Perch	22.5	MARG	1	N	0.26	5.08E-01	6.92E-01	1.08
La Prairie	Perch	21.5	MARG	1	N	0.26	1.85E+00	2.73E-01	1.26
La Prairie	Perch	12.5	POS	6	N	3.00	3.42E-01	2.62E-01	2.32
La Prairie	Perch	12.5	MARG	1	Y	3.00	1.19E-01	2.26E+00	1.27
La Prairie	Pike	33.0	MARG	2	N	1.56	1.69E-01	1.68E+00	1.23
La Prairie	Pike	55.5	MARG	2	N	1.10	4.80E-01	5.28E-02	1.14
La Prairie	Pike	33.0	MARG	2	N	1.56	1.49E+00	.	1.55
Longueuil	Perch	24.0	POS	6	N	3.00	7.04E-02	3.15E-01	1.09
Longueuil	Perch	11.0	MARG	1	N	3.80	3.40E+00	4.75E-02	1.11
Longueuil	Perch	23.0	POS	4	N	7.04	3.31E+00	1.42E-02	1.28
Longueuil	Perch	23.0	MARG	2	N	7.04	8.06E-01	9.41E-02	1.13
Longueuil	Pike	38.5	MARG	1	Y	1.53	1.17E-01	1.41E+00	1.08
Montreal-East	Perch	23.0	MARG	1	N	1.90	8.97E-01	1.97E-01	1.12
Montreal-East	Perch	23.0	MARG	2	N	1.90	1.81E+00	2.10E-01	1.28
Montreal-East	Perch	24.8	POS	4	N	2.20	1.62E+00	9.88E-02	1.46
Montreal-East	Pike	33.8	POS	3	N	0.41	2.00E-01	1.05E-01	1.26
Montreal-East	Pike	33.8	POS	5	N	0.41	2.63E-01	2.91E-01	1.33
Montreal-East	Pike	58.9	POS	5	N	1.90	1.28E-01	8.45E-01	1.77
Montreal-East	Pike	58.9	MARG	1	Y	1.90	1.19E-01	5.52E+00	1.11
Pt.-aux-Trembles	Perch	26.5	POS	6	N	0.77	4.17E-02	1.38E+00	1.70
Pt.-aux-Trembles	Perch	26.5	POS	5	N	0.77	2.49E-01	7.87E-01	1.66
Pt.-aux-Trembles	Perch	24.5	POS	6	N	0.65	2.68E-02	2.15E+00	1.70
Pt.-aux-Trembles	Perch	26.0	POS	4	N	1.65	3.66E-01	3.81E-01	1.80
Pt.-aux-Trembles	Perch	26.5	MARG	1	Y	0.77	6.11E-02	7.94E-03	1.12
Pt.-aux-Trembles	Perch	24.5	MARG	1	Y	0.65	2.40E-02	1.52E+01	1.12
Pt.-aux-Trembles	Perch	26.0	MARG	2	Y	1.65	3.52E-02	2.98E+00	1.18
Pt.-aux-Trembles	Sauger	38.0	POS	3	N	2.30	5.14E-01	5.23E-01	1.40
Pt.-aux-Trembles	Sauger	38.0	MARG	1	Y	2.30	1.78E-01	2.27E+00	1.06
Sorel	Perch	13.0	MARG	1	N	3.42	3.86E-01	2.71E-01	1.06
Sorel	Perch	17.0	POS	6	N	2.30	7.90E-02	6.40E-02	1.70
Sorel	Perch	14.8	POS	6	N	1.80	2.79E-01	.	2.24
Sorel	Perch	13.0	POS	4	N	3.40	9.18E-01	6.90E-02	1.97
Sorel	Perch	11.0	POS	5	N	3.60	1.04E+01	3.36E-02	1.47
Sorel	Perch	17.0	MARG	1	Y	2.00	8.22E-02	4.08E+00	1.13
Sorel	Perch	14.8	POS	3	Y	1.80	2.04E-01	7.64E-01	1.72

Sorel	Pike	56.5	MARG	1	N	1.13	5.39E-02	2.80E+00	1.07
Sorel	Pike	8.5	MARG	1	N	0.75	9.15E-02	7.12E+00	1.32
Sorel	Pike	8.5	MARG	1	Y	0.75	2.80E-01	4.20E+00	2.66
St. Romuald	Perch	26.0	POS	5	N	1.25	6.34E-02	2.91E-01	1.52
St. Romuald	Perch	24.0	POS	5	N	1.10	2.32E-02	3.50E-01	.
St. Romuald	Perch	25.5	POS	3	N	1.04	1.03E-02	3.90E+00	.
St. Romuald	Perch	26.0	MARG	2	Y	1.25	3.80E-02	2.92E+00	1.17
St. Romuald	Walleye	38.5	POS	6	N	0.21	1.43E-02	4.76E-01	1.47
St. Romuald	Walleye	22.2	ERRATIC	3	N	1.20	.	.	.
Tracy	Perch	16.5	POS	4	N	1.50	3.68E-01	4.71E-01	1.24
Tracy	Perch	16.5	POS	4	N	1.50	1.99E+00	1.15E-01	1.36
Tracy	Perch	5.5	POS	4	N	4.85	2.59E+01	.	1.32
Tracy	Perch	15.5	POS	5	N	2.70	1.35E-01	3.33E-01	1.60
Tracy	Perch	5.5	POS	3	N	4.85	1.30E+00	6.92E-01	1.80
Tracy	Perch	19.8	POS	6	N	3.20	7.62E-01	1.36E-01	1.81
Tracy	Perch	5.5	POS	6	N	4.85	1.96E+00	.	1.28
Tracy	Perch	5.5	MARG	1	Y	4.85	3.56E-01	6.59E-01	1.23
Tracy	Perch	15.5	MARG	1	Y	2.70	6.23E-02	2.87E+00	1.09
Tracy	Perch	19.8	MARG	2	Y	3.20	1.68E-01	6.61E-01	1.42
Tracy	Pike	85.5	POS	5	N	1.90	1.17E+00	2.70E-01	1.85
Trois Rivières	Perch	19.2	MARG	1	N	4.00	6.76E-02	1.19E+00	1.16
Trois Rivières	Perch	13.3	ERRATIC	3	N	3.90	.	.	.
Trois Rivières	Pike	43.0	POS	4	N	0.47	2.96E-02	1.15E+00	1.08
Trois Rivières	Pike	38.0	POS	5	N	1.03	7.56E-03	1.17E+00	1.18
Trois Rivières	Pike	52.5	MARG	2	N	0.70	2.58E-02	3.10E+00	1.05
Varennes	Perch	15.7	POS	6	N	1.63	3.15E-01	2.86E+00	1.92
Varennes	Perch	23.7	POS	4	N	1.70	2.53E+00	8.76E-02	1.46
Varennes	Perch	16.6	MARG	2	N	1.75	1.19E+00	1.20E-01	1.10
Varennes	Perch	16.9	MARG	1	N	3.30	4.15E-01	4.74E-01	1.11
Varennes	Perch	15.7	MARG	1	N	1.63	1.52E+00	1.01E-01	1.13
Varennes	Perch	15.7	MARG	2	Y	1.63	1.10E-01	1.37E+00	1.41
Varennes	Pike	35.1	MARG	2	N	0.36	1.24E+00	2.94E-01	1.45
Varennes	Pike	33.9	MARG	2	N	0.40	5.89E-01	4.21E-01	1.28

Pike or Northern Pike = *Esox lucius*

Perch or Yellow Perch = *Perca flavescens*

Walleye or Yellow Walleye = *Stizostedion vitreum*

Sauger or Sand Pickerel = *Stizostedion canadense*

Herring or Atlantic Herring = *Clupea harengus*

Trout or Brook Trout = *Salvelinus fontinalis*
 Smelt or Rainbow Smelt = *Osmerus mordax*

2) Genotoxicity of macroinvertebrate tissue extracts.

Variables:

Sample = Type of invertebrate collected (see below).

Resp. = SOS Chromotest result.

Above = Number of concentrations that exceeded control.

Activ. = S9 Activation status.

Lipid = Lipid content (% wet tissue wt.).

SRIP-Wet = Genotoxic potency in IF Units per equivalent g of wet tissue.

LOGC-Wet = Lowest observable genotoxic concentration in equivalent g wet tissue per assay mL.

MaxIF = Maximum Induction Factor observed

Site	Sample	Resp.	Above	Activ.	Lipid	SRIP-Wet	LOGC-Wet	MaxIF
Beauharnois	Mix Benth	POS	3	N	0.60	1.99E+00	7.04E-02	1.17
Beauharnois	Mix Benth	MARG	1	N	0.60	1.05E+00	1.28E-01	1.22
Beauharnois	Mix Benth	POS	6	N	0.33	2.36E-01	.	1.46
Beauharnois	Profund	MARG	1	N	0.20	1.41E-01	1.79E+00	1.17
Beauport	Gam/Cray	MARG	1	Y	0.37	9.54E-02	2.07E+00	1.29
Beauport	Gastropod	POS	3	N	0.06	6.94E-02	1.25E+00	1.16
Beauport	Gastropod	MARG	2	Y	0.05	1.80E-02	1.10E+01	1.16
Bécancour	Gam/Gast	POS	3	N	0.42	1.39E-02	3.41E+00	1.24
Bécancour	Gastropod	MARG	1	Y	0.10	2.05E-01	.	2.30
Bécancour	Gastropod	POS	4	N	0.10	2.07E-01	1.18E+00	1.50
Bécancour	Gastropod	ERRATIC	4	N	0.18	.	.	1.14
Bécancour	Gastropod	MARG	1	Y	0.18	2.79E-02	4.43E+00	1.19
Boucherville	Gastropod	MARG	2	N	0.37	1.32E+00	3.48E+00	8.30
Contrecoeur	Gastropod	MARG	2	N	0.24	4.23E-02	1.79E+00	1.40
Contrecoeur	Gastropod	POS	2	N	0.24	5.78E-02	3.63E-01	1.28
Contrecoeur	Gastropod	POS	4	N	0.24	1.41E-01	2.24E-01	1.20
Donnacona	Crayfish	POS	3	N	1.14	.	.	.
Donnacona	Gastropod	POS	5	N	0.13	1.72E-02	1.29E+00	1.41
Donnacona	Gastropod	POS	4	N	0.07	1.79E-02	2.50E+00	1.47
Donnacona	Gastropod	MARG	1	Y	0.07	1.13E-02	2.32E+01	1.26
Longueuil	Mix Benth	MARG	2	N	0.75	1.70E+00	1.56E-01	1.47

Pt.-aux-Trembles	Gastropod	POS	5	N	0.27	7.19E-02	.	1.32
Sorel	Gammarus	MARG	2	N	.	.	.	.
Sorel	Bivalve	POS	3	N	0.28	4.23E-02	2.47E+00	.
St. Romuald	Crayfish	POS	5	N	0.73	3.13E-02	.	1.46
St. Romuald	Crayfish	MARG	1	N	0.98	.	.	1.04
St. Romuald	Crayfish	MARG	1	Y	0.73	2.55E-02	7.76E+00	1.13
Tracy	Crayfish	POS	3	N	0.86	8.23E-02	1.00E-01	1.09
Tracy	Crayfish	POS	4	N	0.86	5.95E-01	1.35E-01	1.37
Tracy	Crayfish	POS	6	N	0.86	4.20E+00	7.76E-02	1.47
Tracy	Crayfish	POS	6	N	0.21	2.05E-01	.	2.30
Tracy	Crayfish	MARG	1	Y	0.21	9.23E-02	2.83E+00	1.31
Trois Rivières	Crayfish	MARG	2	N	.	.	.	.
Trois Rivières	Crayfish	POS	6	N	1.25	2.46E-01	.	1.52
Trois Rivières	Crayfish	POS	3	Y	1.25	1.71E-01	9.38E-01	1.32
Trois Rivières	Gam/Gast	MARG	1	Y	1.21	1.09E-01	4.76E+00	1.31

Additional information about invertebrates samples collected at sites listed above.

Site	Sample	Description
Beauhamois	Mix Benth	Mixture of: Oligochaeta, Zygoptera, Gammaridae, Physidae.
Beauhamois	Profund	Mixture of mollusks: Bithyniidae, Lymnaeidae, Unionidae, Sphaeriidae.
Beauport	Gam/Cray	Gammarus sp. and Decapoda.
Beauport	Gastropod	Primarily Bithyniidae.
Bécancour	Gam/Gast	Gammarus sp., Bithyniidae and Planorbidae.
Boucherville	Gastropod	Primarily Bithyniidae and Planorbidae.
Contrecoeur	Gastropod	Primarily Bithyniidae and Planorbidae.
Donnacona	Crayfish	Decapoda.
Donnacona	Gastropod	Primarily Bithyniidae and Planorbidae.
Longueuil	Mix Benth	Mixture of: Oligochaeta, Zygoptera, Gammaridae, and Hemiptera.
Pt.-aux-Trembles	Gastropod	Primarily Bithyniidae, Planorbidae and Physidae.
Sorel	Gammarus	<i>Gammarus sp.</i>
Sorel	Bivalve	Unionidae.
St. Romuald	Crayfish	Decapoda.
Tracy	Crayfish	Decapoda.
Trois Rivières	Crayfish	Decapoda.
Trois Rivières	Gam/Gast	Gammarus sp. and Decapoda.

3) Polycyclic aromatic hydrocarbon data.

Variables:

Total Length = Total fish length (cm). Composite samples are given as range.

Sample = Samples type.

Lipid = Lipid content (% wet wt).

Tot. PAH = Total PAH concentration (ng per g wet wt).

All PAH concentrations are in ppb (ng per g wet wt). 0.00 indicates below detection detection limits are 0.5-1.0 ppb.

PAH Abbreviations are: Naphth = naphthalene, Aceny = Acenaphthylene, Acenaph = Acenaphthene, Fluor = Fluorene, Phenanth = Phenanthrene, Anthra = Anthracene, Fluoranth = Fluoranthene, Pyr = Pyrene, BcFhen = Benzo(c)phenanthrene, BaA = Benz(a)anthracene, Chrys = Chrysene, BbKf =

Benzo(b+k)fluoranthene, DMBA = 7,12-Dimethylbenz(a)anthracene, BaP = Benzo(a)pyrene, MChol = 3-Methylcholanthrene, IndPyr = Indeno(1,2,3-cd)pyrene,

DBahAnt = Dibenz(ah)anthracene, BghiPer = Benzo(ghi)perylene, DBaIPyr = Dibenz(ai)pyrene, DBahPyr = Dibenz(ah)pyrene, DBaIPyr = Dibenz(ai)pyrene.

Site	Total Length	Sample	Lipid	Tot PAH	Naphth	Aceny	Acenaph	Fluor	Phenan	Anthra	Fluoranth	Pyr
Varenes	16.6	Perch	1.75	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Varenes	15.7	Perch	1.63	5.98	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00
Varenes	16.9	Perch	3.30	6.86	0.98	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Varenes	23.7	Perch	1.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tracy	5.5	Perch	4.85	12.90	0.99	0.00	0.00	0.99	0.99	0.00	0.00	0.00
Sorel	10-12	Perch	3.60	2.99	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
Sorel	14.8	Perch	1.80	1.97	0.00	0.00	0.00	0.00	1.96	0.00	0.00	0.00
Sorel	16-18	Perch	2.00	1.98	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00
Sorel	<13	Perch	3.42	3.96	0.00	0.00	0.00	0.99	2.97	0.00	0.00	0.00
Tracy	19.8	Perch	3.20	0.60	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00
Tracy	15.5	Perch	2.70	0.99	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00
Memphre	14.5	Perch	2.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Memphre	18.6	Perch	2.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pt-aux-Tremb	26.5	Perch	0.77	3.99	0.00	0.00	0.00	0.00	2.00	0.00	0.00	0.00
Pt-aux-Tremb	24.5	Perch	0.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pt-aux-Tremb	26.0	Perch	1.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mtl-East	33.8	Perch	0.41	0.99	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00
Mtl-East	13.7	Perch	4.59	2.96	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00
Mtl-East	23.0	Perch	1.90	3.95	0.00	0.00	0.00	0.99	0.00	2.97	0.00	0.00
Mtl-East	24.8	Perch	2.20	0.99	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00
Beauharnois	20.0	Perch	0.53	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Beauharnois	12.0	Perch	2.30	14.88	0.00	0.00	0.00	0.00	1.98	0.00	1.98	0.99
Beauharnois	21.0	Perch	0.54	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Beauhamois	12.5	Perch	2.20	0.97	0.00	0.00	0.00	0.00	0.97	0.00	0.00	0.00
Beauhamois	11.5	Perch	3.33	155.28	1.97	0.00	0.00	8.85	8.85	2.95	0.00	0.00
Longueuil	11.0	Perch	3.80	1.97	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00
Longueuil	23.0	Perch	7.04	1.98	0.00	0.00	0.00	0.00	1.98	0.00	0.00	0.00
Longueuil	11.5	Perch	3.20	14.75	0.00	0.00	0.00	0.00	0.98	0.00	0.00	0.00
Longueuil	24.0	Perch	3.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Longueuil	11.5	Perch	2.90	1.98	0.00	0.00	0.00	0.00	1.98	0.00	0.00	0.00
Longueuil	23.5	Perch	2.80	5.73	0.95	0.00	0.00	1.91	0.95	0.00	0.00	0.00
La Prairie	21.5	Perch	0.26	4.93	1.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00
La Prairie	25.5	Perch	0.77	1.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
La Prairie	12.5	Perch	3.00	4.94	0.00	0.00	0.00	0.00	0.99	0.00	0.99	0.00
Contrecoeur	13.0	Perch	2.60	7.97	0.00	0.00	0.00	0.00	1.99	0.00	0.00	0.00
Contrecoeur	14.0	Perch	3.20	2.97	0.00	0.00	0.00	0.00	2.97	0.00	0.00	0.00
Contrecoeur	24.0	Perch	1.60	2.96	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00
Contrecoeur	34.6	Perch	0.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Contrecoeur	17.0	Perch	3.90	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Contrecoeur	16.0	Perch	3.90	4.00	0.00	0.00	0.00	0.00	4.00	0.00	0.00	0.00
Contrecoeur	17.6	Perch	4.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Contrecoeur	23.5	Perch	2.80	1.99	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00
Boucherville	26.0	Perch	2.00	0.99	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00
Boucherville	13.9	Perch	3.68	3.91	1.95	0.00	0.00	0.00	0.98	0.00	0.00	0.00
Boucherville	13.5	Perch	4.50	13.84	0.00	0.00	0.00	1.98	10.87	0.00	0.99	0.00
Boucherville	14.5	Perch	4.32	25.62	6.90	0.00	0.99	6.90	4.93	1.97	0.00	0.00
Boucherville	25.5	Perch	1.86	3.98	0.99	0.00	0.00	0.99	1.99	0.00	0.00	0.00
Boucherville	26.5	Perch	0.59	1.89	0.94	0.00	0.00	0.00	0.94	0.00	0.00	0.00
Varennes	40.5	Pike	.	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Varennes	33.9	Pike	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Varennes	35.1	Pike	0.36	0.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sorel	56.5	Pike	1.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sorel	8-12	Pike	0.75	5.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
Tracy	51.5	Pike	0.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tracy	85.5	Pike	1.90	0.99	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00
Tracy	10.0	Pike	0.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mtl-East	58.9	Pike	1.90	15.50	0.00	0.00	0.00	0.00	1.94	0.00	0.97	1.94
Beauhamois	50.0	Pike	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Longueuil	48.0	Pike	0.36	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Longueuil	38.5	Pike	1.53	5.93	0.99	0.00	0.00	0.99	0.00	0.99	0.00	0.00
La Prairie	33.0	Pike	1.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
La Prairie	55.5	Pike	1.10	1.93	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Contrecoeur	38.8	Pike	0.76	2.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Contrecoeur	33.0	Pike	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Boucherville	54.5	Pike	0.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Boucherville	49.9	Pike	0.91	1.97	0.00	0.00	0.00	0.00	1.97	0.00	0.00	0.00
Pt-aux-Tremb	34.0	Sauger	2.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pt-aux-Tremb	38.0	Sauger	2.30	4.96	0.00	0.00	0.00	0.00	2.98	0.00	0.00	0.00
Pt-aux-Tremb	Invert	Mixed	1.02	17.14	0.00	0.00	0.00	0.00	2.86	0.00	2.86	4.29
Longueuil	Invert	Mixed	0.75	30.84	1.99	0.00	0.00	0.00	2.98	0.00	5.97	3.98
Tracy	Invert	Crayfish	0.86	2.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00
Tracy	Invert	Crayfish	0.21	7.82	0.98	0.00	0.00	0.00	1.96	0.00	0.00	0.00
Sorel	Invert	Gammar	0.56	12.92	0.00	0.00	0.00	0.99	3.98	3.98	0.99	0.99
Sorel	Invert	Gam/Cray	0.32	32.84	0.94	0.00	0.00	0.00	5.63	0.00	10.32	6.57
Tr Rivières	Invert	Gam/Gast	1.21	159.53	1.95	0.97	1.95	0.00	15.56	0.00	1.95	3.89
Boucherville	Invert	Gam/Gast	0.63	22.71	0.00	0.00	0.00	0.00	4.73	0.00	3.78	3.78
Pt-aux-Tremb	Invert	Gastropod	0.27	16.76	1.97	0.00	0.00	0.00	3.94	0.00	2.96	2.96
Contrecoeur	Invert	Gastropod	0.24	2.99	1.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Boucherville	Invert	Gastropod	0.37	19.48	0.00	0.00	0.00	0.00	3.90	0.00	4.87	0.00
Bécancour	Invert	Hirudinea	0.15	279.21	0.99	0.00	0.99	0.00	14.85	2.97	60.40	39.60
Beauharnois	Invert	Mixed	0.60	216.89	0.96	0.00	0.00	0.96	15.42	4.82	37.59	28.92
Beauharnois	Invert	Mixed	0.33	73.98	0.99	0.00	0.00	0.00	4.93	0.00	10.85	6.90
La Prairie	Invert	Mixed	0.75	15.91	0.00	0.00	0.00	0.00	1.99	0.00	3.98	2.98
Mtl-East	Invert	Mixed	0.48	52.48	0.00	0.00	0.00	0.00	2.92	0.00	5.83	9.72
Contrecoeur	Invert	Mixed	1.43	6.91	0.99	0.00	0.00	0.00	2.96	0.00	0.99	0.99
Tracy	Invert	Mixed	0.86	15.56	0.97	0.00	0.00	0.00	2.92	0.00	1.95	0.97
Sorel	Invert	Unionid	0.65	67.93	0.98	0.00	0.00	0.00	4.92	0.00	20.67	21.56
Beauharnois	Invert	Profund	0.20	69.57	0.00	0.00	0.00	0.00	3.92	0.98	8.82	7.84

	BcPhen	BaA	Chrys	BbjkF	DMBA	BaP	MChol	IndPyr	DBahAnt	BghiPer	DBaiPyr	DBahPyr	DBalPyr
Varenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Varenes	0.00	1.00	3.98	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Varenes	0.00	0.00	5.88	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Varenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tracy	0.00	0.99	8.93	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sorel	0.00	0.00	1.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sorel	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sorel	0.00	0.00	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sorel	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tracy	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Tracy	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Memphre	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Memphre	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pt-aux-Tremb	0.00	0.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pt-aux-Tremb	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pt-aux-Tremb	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mtl-East	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mtl-East	0.00	0.00	1.98	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mtl-East	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mtl-East	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Beauhamois	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Beauhamois	0.00	0.00	6.94	2.98	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Beauhamois	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Beauhamois	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Beauhamois	0.00	8.85	117.94	0.00	0.00	5.90	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Longueuil	0.00	0.00	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Longueuil	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Longueuil	0.00	0.98	12.78	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Longueuil	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Longueuil	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Longueuil	0.00	1.91	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
La Prairie	0.00	0.00	2.96	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
La Prairie	0.00	1.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
La Prairie	0.00	0.00	2.96	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Contrecoeur	0.00	0.00	5.98	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Contrecoeur	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Contrecoeur	0.00	0.00	1.98	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Contrecoeur	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Contrecoeur	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Contrecoeur	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Contrecoeur	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Contrecoeur	0.00	0.00	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Boucherville	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Boucherville	0.00	0.00	0.98	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Boucherville	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Boucherville	0.00	0.00	3.94	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Boucherville	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Boucherville	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Varenes	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

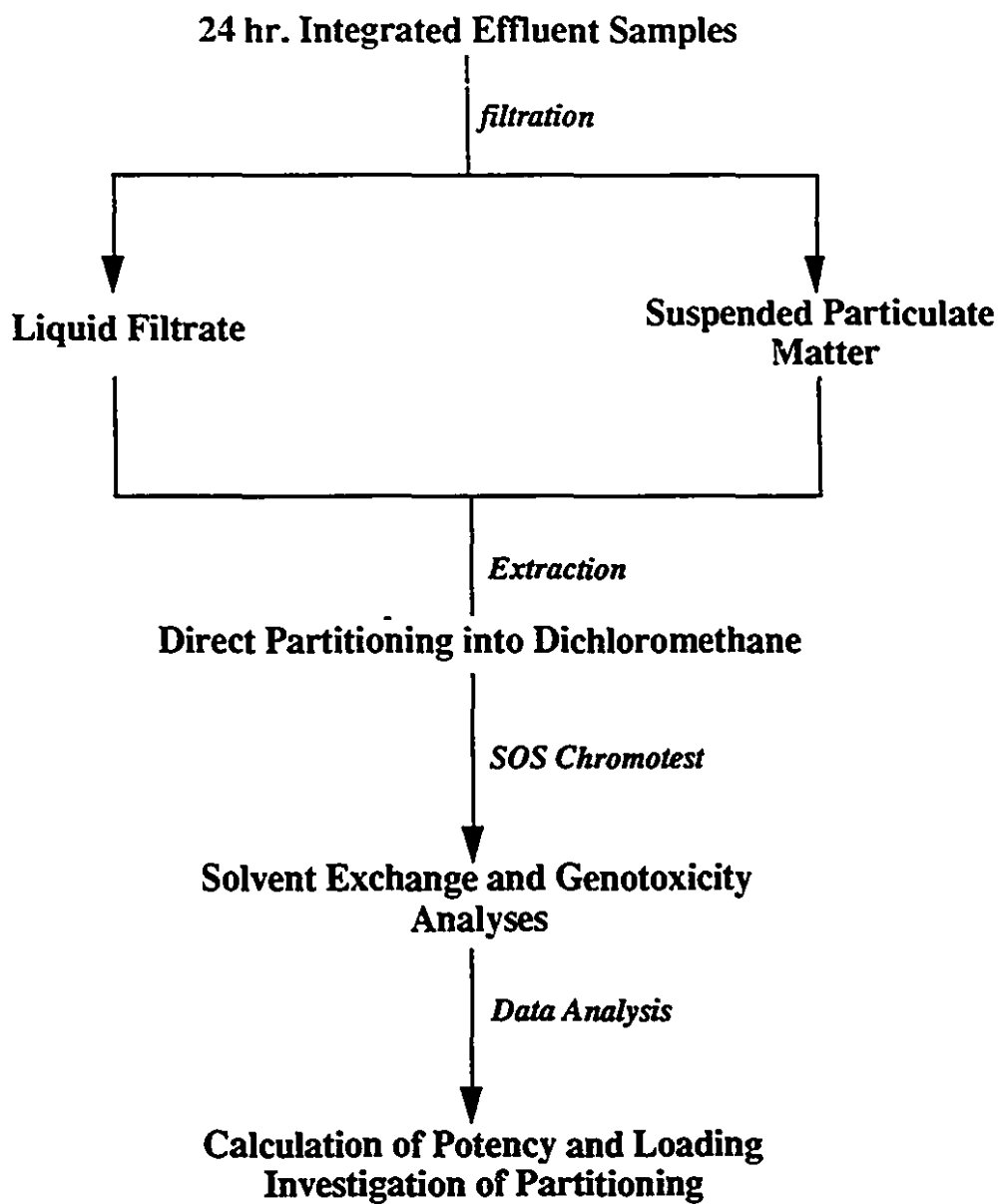
Varennes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Varennes	0.00	0.00	0.00	0.00	0.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sorel	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sorel	0.00	0.00	4.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tracy	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tracy	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tracy	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mtl-East	0.00	1.94	2.91	3.88	1.94	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Beauharnois	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Longueuil	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Longueuil	0.00	0.00	0.99	0.00	0.00	0.00	0.00	1.98	0.00	0.00	0.00	0.00	0.00
La Prairie	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
La Prairie	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.93	0.00	0.00	0.00	0.00
Contrecoeur	0.00	0.00	2.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Contrecoeur	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Boucherville	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Boucherville	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pt-aux-Tremb	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pt-aux-Tremb	0.00	0.00	1.98	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pt-aux-Tremb	0.00	4.29	1.43	1.43	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Longueuil	0.00	0.00	5.97	6.96	0.00	2.98	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tracy	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tracy	0.00	3.91	0.98	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sorel	0.00	0.00	1.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sorel	0.00	2.81	3.75	2.81	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tr Rivières	0.00	21.40	107.00	1.95	0.00	2.92	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Boucherville	0.00	1.89	3.78	3.78	0.00	0.00	0.00	0.00	0.00	0.95	0.00	0.00	0.00
Pt-aux-Tremb	0.00	2.96	1.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Contrecoeur	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Boucherville	0.00	3.90	6.82	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bécancour	0.00	26.73	99.01	28.71	0.00	4.95	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Beauharnois	0.00	21.21	26.99	38.56	0.00	16.39	0.00	7.71	1.93	9.64	1.93	0.96	2.89
Beauharnois	15.78	18.74	3.95	11.84	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
La Prairie	0.00	2.98	1.99	1.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mtl-East	0.00	4.86	9.72	7.77	0.00	6.80	0.00	1.94	0.00	2.92	0.00	0.00	0.00
Contrecoeur	0.00	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tracy	0.00	2.92	2.92	1.95	0.00	0.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sorel	0.00	3.94	12.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.95
Beauharnois	0.00	5.88	13.72	17.64	0.00	3.92	0.00	2.94	0.00	3.92	0.00	0.00	0.00

APPENDIX 11

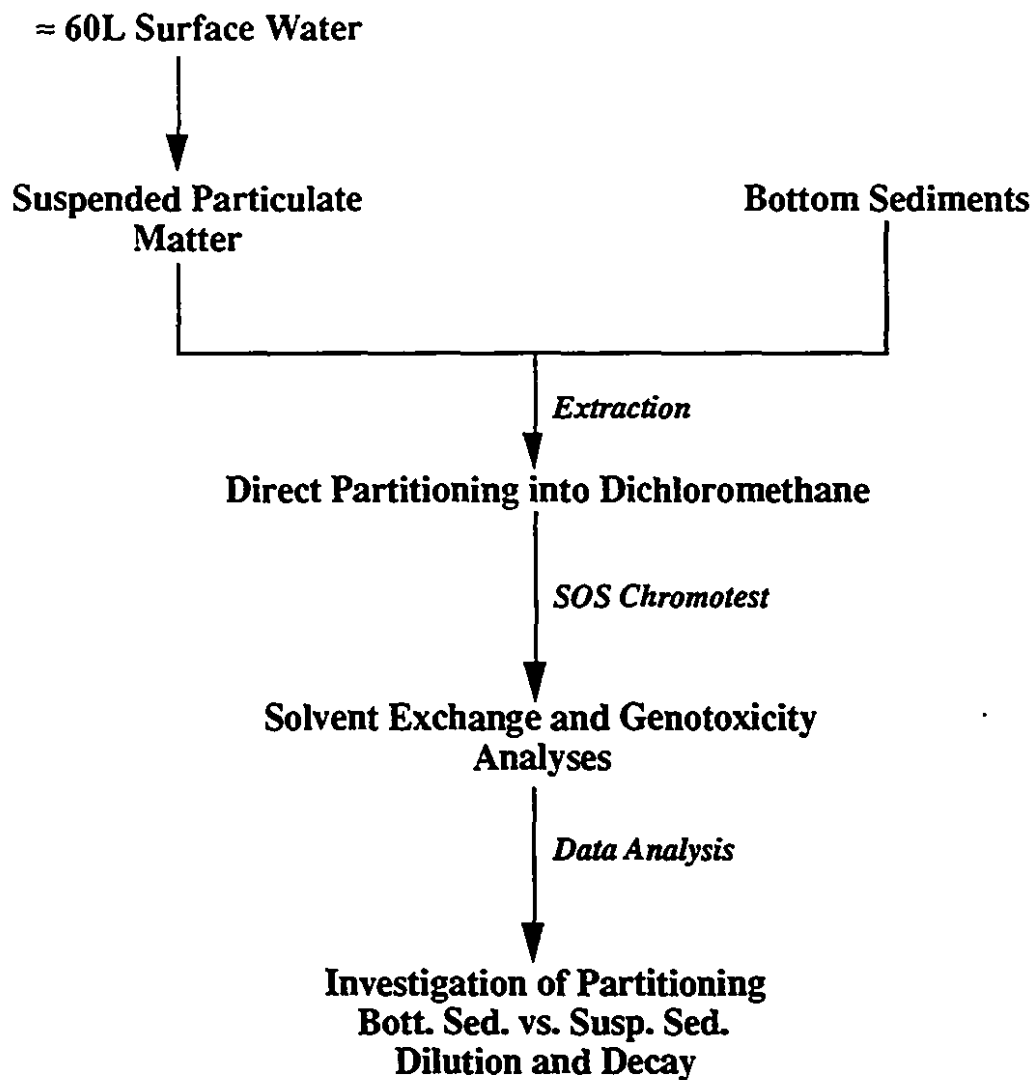
Flow Charts of Sample Handling

Pursuant to the request of the internal examiner, flow charts of sample handling have been included in the final version of the thesis.

1) Handling of effluent samples examined in Chapters 3 and 4--



2) Handling of samples examined in Chapter 5--



3) Handling of samples examined in Chapters 7 and 8--

