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THE FUTURE OF GENOMICS FOR ECOSYSTEM RESEARCH AT NWRI

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Management Perspective

Scientist and research managers at NWRI have identified several key areas of environmental research that will be impacted by the advances in genetics and molecular biology. This report is a compilation of the comments and recommendations reached by the Molecular Biology Planning Group regarding the impact that molecular genetics is having, or will have, on NWRI research programs.

Sommaire à l'intention de la direction

Les scientifiques et les directeurs de recherche de l'INRE ont dressé une liste des principaux secteurs de recherche environnementale qui devraient bénéficier des percées réalisées dans les domaines de la génétique et de la biologie moléculaire. Ce rapport résume les commentaires et recommandations formulés par le groupe de planification des recherches en biologie moléculaire concernant l'impact actuel et éventuel de la génétique moléculaire sur les programmes de recherche de l'INRE.

Abstract

Molecular genetics is a rapidly advancing discipline, and repercussions from this branch of science have penetrated virtually every discipline in the natural sciences. Scientists and research managers at NWRI have identified several areas of environmental research that are likely to be impacted by the advances in genetics and molecular biology, including: environmental toxicology, pathogens in the environment, biodiversity, the impact of genetically modified organisms, analytical chemistry, and bioremediation. Research in those areas will directly impact Environment Canada's mandates in two business lines: Clean Environment and Nature. To further discussion and progress on this topic at NWRI, a planning committee was organized, and tasked with assessing the influence that molecular genetics will have on environmental research at NWRI. This report is a compilation of the comments and recommendations reached by the Molecular Biology Planning Group.

Résumé

La génétique moléculaire est une discipline en plein essor, et les percées réalisées dans ce domaine ont un retentissement sur pratiquement toutes les disciplines liées aux sciences naturelles. Les scientifiques et les directeurs de recherche de l'INRE ont dressé une liste des principaux secteurs de recherche environnementale qui devraient bénéficier des percées réalisées dans les domaines de la génétique et de la biologie moléculaire. Cette liste inclut la toxicologie environnementale, l'étude des pathogènes dans l'environnement, l'étude de la biodiversité, l'évaluation des impacts des organismes génétiquement modifiés, la chimie analytique et la biorestauration. Les recherches entreprises dans ces domaines auront un impact direct sur les mandats d'Environnement Canada dans deux secteurs d'activité, à savoir l'assainissement de l'environnement et la protection de la nature. Pour promouvoir la discussion sur cette question et évaluer les progrès réalisés dans ce domaine à l'INRE, un comité de planification créé pour la circonstance a été chargé d'évaluer l'incidence de la génétique moléculaire sur la conduite des recherches touchant l'environnement à l'INRE. Ce rapport résume les commentaires et recommandations formulés par le groupe de planification des recherches en biologie moléculaire.

EXECUTIVE SUMMARY

Molecular genetics is a rapidly advancing discipline, and repercussions from this branch of science have penetrated virtually every discipline in the natural sciences. Recent biotechnological advances in the field of genetics have reached an unprecedented level. The genomes from numerous organisms have been completely sequenced, and many more will soon follow. Sophisticated molecular tools enable researchers to examine the response of *all* genes in an organism, paving the way to global mapping of gene expression and complete characterization of cellular and biochemical networks. Other techniques provide efficient methods of shuffling genes among and between organisms. These advances and concomitant discoveries will drastically impact the research and assessment of environmental health.

Genetic-based biotechnology has become important to the public. A recent survey published in *Nature Biotechnology*, a leading scientific journal in the field, revealed that only 8.8% of Canadians strongly felt that current federal regulations were sufficient to protect the public from risks that may result from genetic activities such as cloning and genetic modification of organisms (*1*). It is projected that public concern over biotechnology advances will become increasingly "precautionary" (*2*), and research directed at assessing the risks that biotechnology posses to human and environmental health will become increasingly important (*2*).

Scientists and research managers at NWRI have identified several areas of environmental research that are likely to be impacted by the advances in genetics and molecular biology: environmental toxicology, pathogens in the environment, biodiversity, the impact of genetically modified organisms, analytical chemistry, and bioremediation. Research in those areas will directly impact Environment Canada's mandates in two business lines: Clean Environment and Nature (see Table 1. Relevance of genomics-based research to Environment Canada's business lines at NWRI.).

To further discussion and progress on this topic at NWRI, a planning committee was organized, and tasked with assessing the influence that molecular genetics will have on environmental research at NWRI. A Molecular Biology Planning Group meeting was convened on March 1, 2001. At the meeting, presentations were given by NWRI scientists and University professors. The presentations emphasized the relevance of genetics-based molecular research in ecosystem health assessment, and highlighted areas of environmental research currently using molecular tools. The impacts that genomics research will have on NWRI programs were considered, and approaches to building molecular biology research capacity were also discussed.

In summary, the planning committee concluded and made the following recommendations:

- There is a unanimous agreement that molecular biology tools will be instrumental in strengthening research capacity, capability, productivity, and the overall impact of NWRI's research.
- NWRI's recognition as a world class institute will be enhanced by participation in the development of genetic tools, and using them to complement the institute's current

research programs. It is inevitable that highly advanced molecular tools will become standard laboratory assays in many areas of ecosystem health research, and that moving proactively in this area would establish a presence and expertise for NWRI. Failure to become active in genomics research may severely affect the overall impact of our science in many areas of environmental research.

- Genomics research is also needed to provide the knowledge and data on which scientific risk assessments can be based. Environmental research and risk assessment will positively influence public concerns over biotechnology issues.
- Genomics research capacity needs to be developed at NWRI. An in-house genomics facility would provide flexibility in the development and use of molecular tools in several areas directly applicable to NWRI research mandates. The 'high-tech' nature of this area means the products or techniques developed will likely become marketable commodities. NWRI may encounter legal issues related to patents and unrestricted use or access to the products developed in collaboration with external research agencies.
- It is essential that NWRI scientists develop 'hands-on' expertise. Without expertise in genomics research applications, scientists cannot properly fulfill their requirements to do research, evaluate grant proposals, review research papers, or provide departmental advice on genomics-related issues. Lack of expertise will also affect the ability of research scientists to critically evaluate the scientific findings now required under CEPA for regulating the development and release of new products related to living organisms (e.g. GMOs).
- Development of genomics capacity need not be at the expense of other research programs. It is emphasized that genomics tools will *complement* many existing programs, and as such, continued support of such areas will assure the full research potential is realized. Multidisciplinary research efforts, in which genomics and bioinformatics can be incorporated, will yield the highest impact science.
- A central *user* facility needs to be developed at NWRI. A user facility approach would reduce the overall cost required to equip individual researchers. The amount of money requested to purchase equipment for immediate applications is about $572,000. An additional $250,000 would be required the following year to purchase a DNA sequencer for additional state-of-the-art capacity.
- The central user facility should be staffed by two highly trained individuals; possibly a senior technician or junior professional (to run and maintain equipment) and a bioinformatics specialist (to collect, analyze, and interpret data). Those individuals would also act as resources for scientists who wish to develop genomics aspects to research projects. A bioinformatics specialist could also be involved in future initiatives to develop a web-based data information system centered, maintained, managed, and developed at NWRI. This is in line with other Federal Government and NWRI initiatives such as CISTI (Canadian Institute for Scientific and Technical Information) and the proposed Canadian Water Research Network (CWRN). Total salaries for the central user staff would be about $150,000.
- A future research position in genetic biodiversity should be considered. Research expertise in this area is non-existent at NWRI. Genomics applications are extensively used as robust tools for assessing biodiversity issues.
- Some studies will require significant product or technique development. NWRI should

consider support of this aspect of research through both capital and research funding investment. The future applications of the end products justify the investments into research development.

- Procurement of the necessary equipment in FY 2001-02 will help scientists initiate genomics-based research without delay. That is critical, since key federal funding initiatives, such as STAGE (Strategic Application of Genomics in the Environment), TSRI (Toxic Substance Research Initiative), EMBRR (Environmental Management of Biotechnology Regulation and Research) and CBS (Canadian Biotechnology Strategy) will come up for renewal in the following fiscal year.

Each of the foregoing issues is dealt with in more detail in this report. The report reviews the use of molecular biology tools in current research programs at NWRI and the importance in developing these tools to help assess ecosystem health issues. A significant portion of the report focuses on the development of microarray technology. Microarray technology is the current pinnacle of molecular biology tool development and provides researchers with the capacity to do cutting-edge, sophisticated, and large scale sample/data collection. Microarrays are powerful tools that have broad applications in research areas related to biodiversity, environmental toxicology, pathogens in the environment, bioremediation, and impact of GMOs.

Biotechnology is a rapidly advancing science. The report also reviews some current trends in biotechnology that will likely raise future challenges for federal government research.

Submitted by the NWRI Molecular Biology Planning Group.

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1. INTRODUCTION

1.1 Background

Since the discovery of DNA as the molecule that encodes life (*3;4*), the field of genetics has experienced significant advances. Repercussions from this branch of science have penetrated virtually every discipline in the natural sciences, and recent technological advances in the field of genetics have reached an unprecedented level. The genomes from 599 viruses, 205 plasmids, 37 bacteria, 1 fungus, 1 plant, and 2 animals have been completely sequenced. Many more will soon follow, including the genome from *Homo sapiens*, a draft sequence of which has recently been published (*5*). Revelations of our genetic structure, and those of other organisms, have challenged the very premises and foundations of biology. Once believed to be fairly static entities, genomes are actually dynamic components, constantly changing, adapting, and evolving.

Molecular technologies have transformed our ability to understand life processes. Mechanistic research has advanced from studies that examine the structure and function of individual genes (or groups of genes), to complex studies that examine the structure, function, and interaction of *all* genes in an organism, paving the way to global mapping of gene expression and complete characterization of cellular and biochemical networks. Genes are easily shuffled from one organism into another (i.e., cloned), or "knocked-out" (i.e., deleted) from the genome entirely. These advances and concomitant discoveries will strongly impact scientific approaches to sustaining the health of our environment.

Genetic tools have created means by which genes can be inserted, removed, shuttled, and propagated within or between different organisms. The result is that our ecosystems are being challenged by an array of genetically modified organisms (GMOs), with Canada being a world-leader in the growth and export of GM crops. Gene manipulation has led to an increase in the numbers of GMOs undergoing development; a trend that is likely to become exponential given recent advances in the molecular biology tools used to develop GMOs (i.e., microarrays, genetic transformation techniques, etc.). A recent survey of European seed producing firms found that in 1999, 33% of seed developers questioned used genetic engineering to construct new variant seeds (2002 estimate is 49%(*6*)). As of the year 2000, almost 29,000 field tests of GMOs have been conducted in the US, with the majority of these GMOs having 'confidential' alterations in genetic structure[1]. Total land use devoted to GMO crops grown internationally increased 11% between 1999 and 2000 (*7*). The development of GMOs is also seen as a new technology to improve aquaculture productivity (*8*) and bio-control of insect-borne diseases. It is difficult, if not impossible, to predict the long-term impact that these GMOs will have on native species, non-target organisms, and their ecologically dependent species. There is recent scientific

[1] Pesticide Action Network. Thousands of field tests of GE crops across the U.S. http://www.panna.org/panna/resources/panups/panup_20010616.dv.html

evidence to suggest that genetically altered organisms may pose a threat to native and non-target species (*7;9*). The potential for GMOs to impact whole ecosystem food webs should not be overlooked. It is essential that we try to understand principles of gene flow and genetic drift within and between populations of organisms, in an attempt to assess the threat that GMOs may pose to ecosystem health.

Genetic tools have also provided scientists with powerful methodologies to assess the health of ecosystems. Many molecular tools are viewed as robust assays for assessing population diversity, species identification, contaminant responses, environmental effects assessments, and gene flow. Together with conventional approaches for assessing ecosystem health, molecular tools can provide a powerful multidisciplinary aspect to environmental health research.

1.2 The Public and Advances in Genetics

A recent survey published in *Nature Biotechnology*, revealed that only 8.8% of Canadians strongly felt that current federal regulations were sufficient to protect the public from risks that may result from genetic activities such as cloning and genetic modification of organisms (*1*). It has been projected that public concern over biotechnology will become increasingly 'precautionary' (*2*). Public interest is determined by broader conceptual (i.e., morality, culture) and political contexts in which biotechnology advances are perceived (*2*), unlike scientific interest which focuses specifically on hypothesis testing, applications, advancement, and individual technologies. This trend in public concern is also reflected in the US and Europe (*10;11*). Investment into research directed at risk assessment of genetic biotechnology is likely to promote public confidence in the government's responsibility to protect and conserve human and environmental health.

1.3 Rationale

Molecular biology will be instrumental in strengthening the research capacity, productivity, and overall impact of NWRI's science. NWRI's international recognition will be enhanced by proactive development and use of these genetic tools. It is inevitable that highly advanced molecular tools will become standard laboratory assays in ecosystem health research. Moving towards this area will establish NWRI's presence and expertise. Failure to pursue these tools may severely affect the overall impact of our scientific contribution in many areas of environmental research in the near future.

This opinion accords with views expressed in the scientific community, and has been a topic of recent discussion in a *Nature* article entitled; "Are You Ready for the Revolution?" (*12*). The article discusses a futuristic approach to scientific research, and emphasizes that multidisciplinary teams will produce the most effective and productive science. High-impact multidisciplinary research will depend on genomics and bioinformatics.

> "...those who learn to conduct high-throughput genomic analyses, and those who can master the

> computational tools needed to exploit biological databases, will have an enormous competitive advantage. Some experts even predict that the outcome of this natural selection will see many current top scientists, research groups, and even whole institutes relegated to the second division."(*12*).

The article adds further emphasis by suggesting that research grant proposals are currently being rejected because they lack a bioinformatics or genomics component. Future research funding will likely be directed towards multidisciplinary research efforts in which aspects of genomics or bioinformatics will be essential.

Although genomics and bioinformatics will impact the research done at NWRI, they are not a 'threat' to current research agendas. Genomic and bioinformatic tools will greatly facilitate and complement many components of NWRI's research programme. Used alone, they will be inadequate to answer complex ecosystem health questions. It is imperative that molecular tools be developed and used within the framework of existing and future research approaches to environmental health. Research at NWRI would be best served by a holistic approach in which genomics, proteomics, physiology, chemistry, and ecology can unite to assess the true health of our ecosystems in a multidisciplinary manner.

1.4 DNA microarrays: cutting edge molecular technology

A significant portion of the report focuses on the development of microarray technology at NWRI. Microarrays are the pinnacle of molecular technology. Acquisition of this technology will provide researchers with the capacity to do cutting-edge, sophisticated, and large scale sample analysis in their research. Microarrays are powerful tools that are having broad applications in research areas related to biodiversity, environmental toxicology, pathogens in the environment, bioremediation, analytical chemistry, and the environmental impact of GMOs.

DNA microarrays are defined as an ordered arrangement of multiple DNA probes fixed to an immobilized surface. More than 100,000 DNA spots, ranging from 70-100 microns in diameter, can be spotted onto a single microscope slide. The two most important types of DNA microarrays are oligonucleotide and PCR fragment microarrays (amplicons). The major underlying rationale for microarrays is to combine the ability to analyze vast numbers of genes with the concept of parallel data acquisition. This parallel processing power allows experimental designs which are much less costly, time-consuming, and produce significantly more data than conventional molecular methods.

Microarrays provide the means to assess the expression of virtually all genes in an organism in response to a stimulus, paving the way to global genetic expression profiling of organisms. Microarrays are commonly being used to characterize genetic diversity, identify biochemical pathways, diagnose disease, characterize drug responses, identify new drug targets, and discover new genes.

More recently, microarray technology has moved into the area of proteomics. Like DNA, individual proteins can be coupled onto a single microscope slide at high spot densities. Protein arrays are currently being used to elucidate signal transduction mechanisms, characterize intracellular biochemical pathways, identify protein targets of drugs and environmental contaminants, and analyze antibody responses against diverse protein targets.

1.5 Genomics-based Research and Environment Canada's Business Lines

Genomics-based research has, and will continue to impact several areas of environmental research. Six key areas of environmental science are likely to be impacted by the advances in genetics and molecular biology. These include biodiversity, environmental toxicology, pathogens in the environment, the impact of genetically modified organisms, analytical chemistry, and bioremediation. Research in these areas is relevant to Environment Canada's mandates in two business lines: Clean Environment and Nature. A summary of the relevance of genomics-based research as it relates to Environment Canada's mandates for NWRI is given in Table 1.

1.6 Report Format

The remainder of the report focuses on the impact that genomics and bioinformatics will have in the key areas of research. It provides insights into the use of molecular biology tools in current research programs at NWRI and the importance in developing advanced genetic tools to help assess ecosystem health issues.

Biotechnology is a rapidly advancing science. The report also reviews some current trends in biotechnology that will likely raise future challenges for federal government research.

Table 1. Relevance of genomics-based research to Environment Canada's business lines at NWRI.

Business Line	Result	Sub-result	Deliverable
Nature	Human impacts on the health of ecosystems are understood and reduced	Assess and report on current state and trends of ecosystem health	• Research and development of strategies/ tools to assess/ diagnose ecosystem health.
		Advance the understanding of the impacts of human activites on the health of ecosystems	• Research and develop new integrated assessment approaches to determine cumulative impact of multiple environmental stressors on freshwater ecosystems
		Assess and report on the effect of genetically modified organisms on ecosystem health[a]	-
	Priority ecosystems are conserved and restored	Ecosystems initiative (Great Lakes 2020)	• Assess and manage ecosystem health: develop and implement indicators for assessing ecosystem health and report on the status of the Great Lakes Basin. • Conserve ecologically important areas: support habitat and biodiversity conservation through monitoring and research • Identify and reduce remaining sources of harmful pollutants: conduct targeted monitoring and research to improve understanding of the level, fate, and effects of persistent toxic substances.
Clean Environment	Toxics Result: prevention or reduction of the environmental and human health threats posed by toxic substances and other substances of concern	Problem Identification: Existing substances	• Contribute new knowledge on the occurrence, persistence, fate, and effects of toxic or other priority substances/effluents in Canadian aquatic ecosystems. • Impact of biotechnology products on the environment.. • Contribute new knowledge to Canadian and international risk assessment/management of both existing toxics and new substances. • New knowledge/techniques on the prevention, treatment, and remediation of contamination in aquatic ecosystems.

[a] new initiative for Environment Canada

2. NWRI RESEARCH PROGRAMS LIKELY TO BE IMPACTED BY GENOMICS

2.1 Environmental Risk of Genetically Modified Organisms (GMOs)

2.1.1 Recent Trends

GMOs are defined as organisms derived through artificial genetic manipulation or selection. GMOs can be created passively through selective breeding programs or actively through genetic engineering. They are most commonly associated with gene manipulation; the insertion of a foreign gene (or genes) into a host organism. Global interest in GMOs is focused predominantly on plant species, particularly those economically important in crop development. Recombinant crops have a selective advantage over native species as a result of the insertion of a gene(s) that render the plant predominantly insect or herbicide resistant.

Transgenic crops (crops into which scientists have inserted genes isolated from microbes, animals, or other plants) have become the most popular and widely used GMOs. Canada is the third largest grower of GMOs in the world and devoted over 10 million acres to transgenic crops in 2000. By 2001, more than forty-eight types of genetic modification in crops had been approved by Health Canada for food consumption in Canada. The most popular transgenic crops currently grown by Canadian farms are canola, soybean, corn, potatoes, flax and tomatoes. The crops and related modified traits so far approved or tested in field trials are listed in Table 2.

Table 2. List of genetically modified crop species and their selective advantage.

Plant	Genetic/Phenotypic Advantage
Corn	Strains resistant to corn borers and herbicides
Canola	Herbicide-resistant strains
Potato	Resistant to Colorado potato beetles
Tomato	Slow ripening strains
Squash	Viral-resistance
Soybean	Herbicide-resistant strains
Flax	Herbicide-resistant strains
Cottonseed oil	Insect resistance
Sugar beet	Herbicide-resistant strains

To date, the main purpose for transgenic technology has been to improve crops by making them more resistant to insects or more tolerant of herbicides. Genetic modification can also enhance a crop's value as food by increasing its nutrient or mineral content. Although early transgenic crops have been designed primarily for pest management purposes, the next generation may shift to more resistance to frost, drought and other stressful environmental conditions and to more nutritious, medically relevant, and more appealing food products (antibiotics, vaccine, cosmetics, pharmaceuticals, etc.).

A major driving force for GMO development in the near future will likely be the use of

GM crops for medical applications. GM potatoes have been developed that express hepatitis B surface antigen, *Escherichia coli* enterotoxin, or the capsid protein of Norwalk viruses (*13;14*), and are being tested for their use as vehicles for oral vaccines against infection (*13;14*). Mice fed recombinant potato tubers have significantly higher antibody responses against immunologic challenge to pathogen-derived antigens when compared to controls (*13;14*). Trials with these GM potatoes have been, or are currently being, conducted in humans (*13;14*). The ability of GM crops to be used as a prophylactic measure against infectious disease will have an important influence in promoting research and development of GM crops. Vaccine-based crops have enormous potential for all countries, particularly Third World countries that can not afford traditional vaccine immunotherapies for infectious disease, but that often suffer the greatest morbidity due to disease. However, the greatest impact that these medically-relevant GM crops may have will be on swaying the global public perspective on GMO development. Recent surveys have shown that there is a world-wide growing concern about the use and development of GM crops. Medically-relevant GMO crops may make the biotechnology road more amenable for future GMO research.

Risk assessment of biotechnology is not a new concept. It has been suggested that a number of potential adverse effects may arise from the release of GMOs into the environment. The likelihood of these effects occurring depends on the organism modified, the novel traits introduced by the genetic modification, and the ecosystem that the GMO is released into. Potential adverse effects of release of GMOs into the environment could include:

1. The dispersal of the GMO in the environment through possible increased persistence, invasiveness and competitiveness with native species, which could change the population dynamics of the release site and the surrounding environment.
2. Effect on population dynamics of organisms (including microorganisms, insects, animals, plants, etc.) in the receiving environment through effects on non-target species which may occur directly or indirectly.
3. Effects on biogeochemistry (e.g. changes in nitrogen and carbon recycling affecting organisms which are important in water or soil decomposition processes).
4. Toxicity and allergenicity of products which are made as a result of the genetic modification.
5. Instability of the genetic modification, (e.g. GMO crops containing virus genes may recombine with plant genome or with other plant viruses to generate more virulent isolates or strains).

The situation is further compounded by using genetic modification techniques that enable the transfer of genetic material between unrelated organisms (e.g., from animal to plant, or interspecies modification) which would not cross under natural conditions.

The ecological risk posed directly or indirectly by the release of GMOs in terrestrial and aquatic ecosystems can lead to potentially long lasting impacts. In the case of GM crops, many scientists initially expected that fewer, less-toxic pesticides would be used, which in turn would benefit the environment. However, the long-term effects of GM crops may be more detrimental to the environment than pesticide use in the long term. Pesticides, in most cases, are temporally and spatially limited in their effectiveness (i.e., a herbicide is applied to a particular field [spatial] in a given season [temporal] and which has a specified persistence [temporal]). GMO introgression and invasion are much more difficult to regulate both spatially and temporally. Once GMOs are introduced, gene flow commences (i.e., pollination, conjugation, reproduction, etc.), resulting in potential introgression (transfer and stable integration of a gene) of GM characteristics to closely related species, and subsequent dispersal throughout a population. The

ecological impacts of such release may not manifest themselves immediately, but require lengthy evolutionary processes such as succession, dispersal, and population competition/displacement to ultimately manifest ecosystem impacts. Furthermore, GM crops may represent only a temporal solution to pest and weed management. It is speculated that insect pests and weeds will eventually evolve tolerance mechanisms to compensate for pesticide and herbicide resistance traits modified in GM crops (*7*).

Gene flow and introgression from GMOs into wild species will have important consequences for the conservation of native populations. Over the last decade, much attention in ecological risk assessment has been focused on hybridization as a potential avenue for the escape of transgenes into natural populations. However, risk assessment should not only focus on the gene flow process but should also address the evolutionary consequence; how genetic diversity of natural populations is altered under gene flow from a related species. To this end, experimental risk assessment should deal with the potential fitness effects of transgenes.

Potential ecological impacts depend on existing opportunities for unintended establishment, persistence, and gene flow of an introduced organism (including alien species or GMOs). Each of these, in turn, depends on various components of survival and reproduction of an organism or its hybrids. Another concern about impact of release of GMOs to the environment is the influence on biodiversity of non-target organisms, including plants, animals, or microorganisms in terrestrial and aquatic ecosystem. Biodiversity of a community has been defined as the biological heterogeneity of a system and assessed by the assemblage of species within the community. Artificial-induced selection (i.e., via GMO release) may dramatically upset the delicate inter-dependencies of organisms within a community that ultimately affect the assemblage or composition of all representative organisms within the community.

An issue for the management of GMOs is how to identify those modifications that may lead to or augment introduced characteristics. Conventional tools used for these purposes, such as morphological, physiological and biochemical traits, have been considered inefficient or inappropriate. Molecular biology tools will have important applications for detecting and monitoring plants and animals ranging from alien species to GMOs. It can be envisioned that with new national and international biotechnology regulatory programs being established, (e.g., United Nations Biosafety Protocol), there will be growing public expectations for reliable risk assessments and monitoring programs to prevent harm from environmental releases or transboundary movements of GMOs. Genomics-based tools offer a promising way to help address some risk assessment challenges as well as to ensure that environmental releases and transboundary movements of GMOs and alien species are carefully monitored.

2.1.2 Research at NWRI

Environment Canada has recently proposed a strategy towards research and monitoring of ecosystem effects of GMOs, and have identified four primary areas of direction[2]. These are:

- to improve our understanding of ecological pathways by which ecosystems, wildlife, and diversity are affected

[2] Generating Knowledge to Understand Ecosystem Effects of Genetically-Modified Organisms (GMOs): Strategic Plan. Environment Canada, January 2001.

by biotechnology, including understanding the impacts of gene transfer in the environment, and indirect effects of biotechnology,

- to improve our understanding of cumulative effects of multiple uses of biotechnology,
- to improve capacity to monitor ecosystems to identify effects of biotechnology and to reduce and mitigate them,
- to improve capacity to detect release of GMOs posing potential risks to the environment from industry and from sewage treatment plants.

Several of these issues are being addressed in current research programs at NWRI. One of these studies examines characteristics of gene flow from genetically modified canola crops to wild relatives. The gene for green fluorescence protein (GFP) has been used as an *in vivo* marker, and fused with the *Bt* gene to develop transgenic canola. Canola has many wild related species (most are weeds) in Canadian fields. The amplification of the *Bt* gene in the hybrids and resulting backcross population is being carried out to confirm the presence of the *Bt* gene. The content of *Bt* protein through *Bt*-enzyme-linked immunosorbent assay (ELISA) in hybrids and backcross populations are being analyzed compared to their parents. PCR and Southern blot hybridization are being used to detect the stacking rate of unique transgenes when different genetically altered canola crops are planted side by side on farmers' lands. Transgene sequences can be obtained through companies, researchers, published data and internet bioinformation services. Specific primers or oligonucleotide can be designed to confirm transgene stacking through PCR or DNA chips.

There is no doubt that transgenes in GM canola can be transferred into wild related species through pollen flow. But the fate of transgene flow in the genome of wild species is a question; that is whether a transgene has been introgressed into the genome of wild species and persists in wild population or deleted. Transgenes can be used to hybridize with recipient genomes to confirm the insertion site through techniques such as fluorescence *in situ* hybridization (FISH) and PCR.

Much of this research requires continued product development, and a diverse array of molecular tools are required in order to conduct research in this area. Highlighted below are specific molecular tools, or approaches, that are required to do GMO research.

1. Green Fluorescence Protein (GFP). GFP has been widely used as a biomarker in many aspects of biology. It is well documented that GFP is non-toxic to a wide range of organisms, and transformation into a variety of organisms leads to stable expression and easy detection. NWRI scientists have developed a transgene-GFP construct to identify potential hybrids and transformed plant lines. GFP can be used as an *in vivo* marker to detect and monitor transgene flow.
2. Transgene amplifications through Polymerase Chain Reaction (PCR). PCR is a fundamental tool in molecular biology techniques. If the sequence of a transgene is available, specific primers can be designed to amplify transgene sequences.
3. Fluorescence In Situ Hybridization (FISH). FISH can be used to detect transgene introgression into wild species genome and persistence in the wild population. FISH provides a direct way to confirm whether a transgene is inserted into the genome of wild species and assess stability of transgene genetic behavior in wild species.
4. Microarrays. One of the most widely used applications of DNA microarrays is to compare and differentiate gene expression patterns of an organism under normal and stressful environmental conditions. Impact analysis of GMO release on the changes of gene expression patterns of microorganisms in aquatic and terrestrial ecosystem, would elucidate which genes are turned on under impact of GMOs. The change of gene expression pattern obtained by this technique could be used as a bioindicator to sensitively detect environmental stress, such as herbicide or *Bt* residue. Microarrays can also be used to detect transgene stacking. GMO crops are developed and released by numerous companies with different modified characteristics or transgenes. Therefore, these transgene may be stacked into each other and into the wild related species. DNA microarrays would provide a quick and reliable way to detect many transgenes at one time.

5. Molecular markers, such as Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), and microsatellites can be used to assess the impacts of GMOs on the biodiversity of microorganisms in aquatic and terrestrial ecosystems. The molecular markers developed from these methods represent the polymorphisms at the DNA level among individuals within populations, species, or a community of organisms.

Ecosystems are complex, and not every risk associated with the release of GMOs can be identified. Unknown risks may surface as the intensification, frequency and scale of the introduction increases. Ecological relationships include many cascading and higher order interactions that are intrinsically difficult to test and evaluate for significance at limited temporal and spatial scales. The research initiatives identified above will enable us to develop and increase scientific capacity to provide knowledge and methodology to detect the release of, and to monitor for the effects of, GMO-associated risks to ecosystems.

2.2 Pathogens in the Environment

2.2.1 Recent trends

There are particularly promising applications for molecular biology tools in the area of environmental microbiology; where they offer a basis for enhancing existing culture-based approaches to detecting plant, animal and human pathogens. DNA microarrays can be applied to more fully characterize and monitor complex microbial communities in biotechnology products and processes such as municipal wastewater treatment. DNA microarrays promise to provide efficient, cost effective, and automated assays to detect the presence of pathogens in drinking water, wastewater or sewage effluents. Microarrays can be designed to detect specific pathogens rather than rely on indicator organisms to assess infectious risks.

The potential for disease transmission via the consumption of contaminated drinking water is enormous, stressing the importance of providing safe drinking water to consumers. There are numerous documented cases of large disease outbreaks due to the consumption of contaminated drinking water. In 1993, over 400,000 people were infected with the intestinal protozoan parasite, *Cryptosporidium parvum*, as a result of drinking contaminated water in Milwaukee, USA. The outbreak resulted in at least 50 deaths. In Canada, the recent outbreak of pathogenic *E. coli* in Walkerton, where over 2000 individuals were infected and several died, represents another case of consumption of contaminated drinking water. The task of providing safe drinking water by utilities is complicated by the relatively low abundance of disease causing organisms, sporadic contamination events, and costs associated with identifying a large number of potential pathogens that can be passed via water. Existing culture-based approaches for pathogen detection need to be supplemented by newer molecular methods, since many pathogens exist in, or can enter into, non-culturable states. This has prompted water utility companies to start investigating the use of DNA microarrays as rapid, automated, and cost effective means to sample drinking water for the presence of pathogens. As an example, Lyonnaise des Eaux, a water treatment company in France, in conjunction with bioMerieux and Affymetrix

(biotechnology companies), have invested 10 million dollars into developing this technology[3].

Genotyping applications of microarray technology are similar in the environmental and medical fields. However, in addition to the ability of DNA microarrays to identify infectious agents through genotyping applications, they are also used to characterize host-pathogen associations. A significant focus in medical research has been to characterize cellular events imposed on host cells in response to infection, as well as to characterize the microbes' response against host-mediated immune attack (*15-17*). This approach is being exploited extensively by pharmaceutical companies for the purpose of drug-targeting and drug design. Microarrays are also being used to characterize appropriate (i.e., response leading to resolution of disease) and inappropriate (i.e., response leading to chronic disease) responses to infection, and to characterize antibiotic resistance in microorganisms.

The medical applications of DNA microarrays will also have enormous potential for determining and predicting the outcome of disease in wildlife, particularly as it relates to their ability to respond to microbial challenges in their environment. This approach can also be used to assess whether individuals exposed to environmental contaminants are more susceptible to disease.

2.2.2 Research at NWRI

2.2.2.1 Pathogen Genotyping

DNA microarrays offer a novel approach for simultaneously screening effluents, emissions and wastes for the presence of many pathogens of environmental or human health concern. With funding from Environment Canada's STAGE Program (Strategic Application of Genomics in the Environment), a research collaboration with NRC's Biotechnology Research Institute (BRI) is focused on exploring applications of DNA microarrays for detecting pathogens in municipal wastewaters. The context for this research has been the growing concern about the emergence and spread of infectious diseases in plant, animal and human populations. Most of this concern has arisen from a human health and agricultural perspective; much less attention has been paid to potential threats to aquatic ecosystems and biodiversity.

In the preliminary STAGE project, total microbial DNA/RNA extractions were performed on municipal wastewater samples, and the DNA/RNA extracts were shipped to NRC-BRI for DNA microarray analysis. DNA microarrays were designed to detect the presence of 30 different pathogens in wastewater samples (17 bacteria, 8 virus families, 4 protozoans, and 1 fungus). Two different DNA microarray designs were compared: one using immobilized oligonucleotides and one using PCR amplicons. The oligonucleotide microarray design used available species-specific probes (e.g. 16S rRNA; heat shock protein - *hsp*60 or *cpn*60; or DNA gyrase - *gyr*B) and toxin-specific probes with a length of 30 nucleotides as a compromise between stringency and reasonable synthesis cost. For every target organism, at least two different probes were designed and each probe was spotted a minimum of two times on the array to provide redundancy and to minimize a false negative result. A total of 288 oligonucleotide probes were designed. The comparative PCR amplicon microarray was designed with five

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[3] See the website: http://www.suez.fr/thema/english/adn/index_frames.htm

amplicons: one 16S rRNA region of *E. coli* and four *Bacillus thuringiensis* PCR fragments including two 16S rRNA regions, one cry1Ac gene fragment and the orfA of plasmid pHD2. These PCR fragments ranged from 263 to 525-bp in size.

Preliminary results have been encouraging, but a number of technical issues still need to be solved in detecting microorganisms and genes in complex environmental samples. Further research is needed for the following reasons: to improve extraction and purification of DNA from wastewater; examine new methods of probe labeling to increase detection sensitivity; explore amplified procedures for universal genes with species-specific DNA sequences for improved bacteria detection; and explore immobilized oligonucleotides for improved viral and toxin gene detection.

2.2.2.2 Biotechnology Product Genotyping

DNA microarrays offer a novel approach to measuring biological diversity. One application of this approach is to provide a better way to characterize biotechnology products and processes composed of complex mixtures of microorganisms. With funding from Environment Canada's EMBRR (Environmental Management of Biotechnology Regulation and Research) Program, research has been initiated with NRC-BRI and the University of Guelph to explore the applications of DNA microarrays for providing a unique genetic 'fingerprint' for microbial consortia products that are subject to notification under CEPA. Complex mixtures of taxonomically unidentified microorganisms (consortia) represent some of the most difficult biotechnology product risk assessment challenges. Consortia products are commercially available in Canada for a range of household uses ranging from drain cleaning to starting up septic tanks.

Consortia present special notification challenges since each one needs to be considered a unique 'substance' under CEPA. There is currently little guidance for characterizing a consortium and determining whether one is equivalent to another. Research is being conducted to design a DNA microarray based upon a variety of taxonomic and functional gene probes that will enable the development of unique genetic fingerprints for microbial consortia products under CEPA. Taxonomic probes will include pathogen probes so that the DNA microarray could also serve as a hazard screening tool. Research will seek to study variation of genetic fingerprints between microbial consortia products to 1) examine the ability to distinguish different consortia products; and 2) monitor microbial constituents of a consortium product between batches for quality control, and over time for determining whether, at some point, it poses new environmental risks and should be considered as a new substance requiring another notification.

2.3 Bioremediation

2.3.1 Recent Trends

The field of bioremediation has progressed rapidly in the last decade and has now reached maturity as a discipline. The remediation of soils and groundwater contaminated by petroleum is largely conducted using aeration and the addition of nutrients to enhance biodegradation. The generally accepted practice has been to induce existing bacterial populations to do the remediation. This approach is favored by the regulators, but is only effective in some

cases. The advent of genomics adds two potential benefits to the field. It provides an unprecedented tool for taxonomic purposes and it opens the avenue for the design of organisms engineered to degrade specific contaminants or to survive in hostile environments.

Many contaminated sites requiring remediation are anaerobic. Highly chlorinated compounds cannot be degraded by aerobic bacteria, but can be attacked by anaerobic consortia. Anaerobic bacteria typically exist in nature in complex consortia or attached to soil/sediment particles, making isolation and culturing of individual strains difficult, and often impossible. The microbial populations present in these environments are classified according to their predominant metabolic pathways. Anaerobic microorganisms are typically characterized indirectly by the distribution of terminal electron accepting processes (TEAPs) such as oxygen, nitrate, iron, or sulphate reduction and methanogenesis (CO_2 reduction). Characterization of environmental microorganisms has been problematic in the past since traditional methods require culturing and isolating pure cultures. It is generally believed that only 1% of environmental microorganisms are culturable. Therefore, it is impossible to study the significance of non-culturable bacterial populations present in nature by using conventional methods.

The advent of molecular biology is dramatically changing taxonomic approaches to classifying and characterizing microbes. Whether the presence of a given bacteria capable of degrading a contaminant, or the effect of a chemical or biological treatment on a population in sediment in groundwater are sought, the use of molecular tools is essential. With molecular biology techniques, a new perspective of bacterial diversity, which is based on genetic and phylogenetic relationships has been established using, DNA/DNA- mRNA/DNA hybridization, PCR, rRNA sequencing, etc. The techniques allow researchers to assess the diversity of bacterial communities without culture of microbes, and has provided researchers with tools to identify and characterize bacteria at the genetic level.

Bioremediation is an area that has significant potential in developing applied applications of basic genomics research. Recent trends in bioremediation have been towards the development of bioabsorptive GMOs. The applications currently focus on the *ex-situ* remediation of metals in soils in enclosed systems. Bioabsorptive GMOs are organisms that that have been genetically engineered to express proteins that minimize the availability of metabolically available forms of a contaminant. A recent paper in *Nature Biotechnology*, describes the cloning of the mouse metallothionein gene (a metal binding protein with high affinity for divalent cations) into a heavy metal resistant bacteria, *Ralstonia eutropha* (*18*). The recombinant protein produced is expressed on the surface of the bacteria and binds Cd^{2+} in contaminated soils. Treatment of Cd^{2+} laden soils with the recombinant bacteria has been shown to reduce the growth-inhibiting toxic effects of Cd^{2+} on tobacco plants.

Recombinant plants containing heavy metal detoxifying enzymes have also been developed. In one study, mercury metabolizing enzymes were inserted in *Arabidopsis thaliana* and outgrowth of the plants determined on mercury-contaminated medium(*19;20*). Genetically engineered plants had better growth rates and detoxified organic mercury to a volatile and much less toxic elemental form (*20*) .

2.3.2 Research at NWRI

Bioremediation research has been conducted over the last decade at NWRI. At the Institute, most of the research can be grouped under the major themes of Ecosystem Effects and

Technology Advancement. In the last re-organization, all remediation activities were grouped in one project, the Aquatic Ecosystem Remediation Project. The majority of the studies in that project either use bioremediation as a method or include an assessment of microbial populations subsequent to a remediation activity.

A list of current studies follows:

- Degradation of chlorinated compounds with vitamin B12 and titanium citrate: effect on *in-situ* microbial populations.
- Biobarriers in fractured bedrock: development and assessment of the microbial population in a biofilm.
- Assessment of the biosafety of using biostimulation and bioaugmentation in the bioremediation of an aquifer contaminated with chlorinated solvents.
- Microbial processes in sediments: avian botulism and algal toxins.
- Bacterial infiltration and transport in groundwater: septic system, intensive farming.
- The role of humic substances in remediation.

The tools that are used differ depending on the hypothesis to be tested. For example, microarrays may be used to verify whether the addition of nutrient sources has led to the predominance of a pathogenic strain, while denaturing gradient gel electrophoresis may be the best method to follow 'population dynamics' over time. In this application, total bacterial DNA is isolated from an environmental sample. PCR is then performed to amplify highly conserved regions of 16S rDNA. DGGE is used to separate these same-sized fragments, based on their melting behaviour in a polyacrylamide gel. Each resulting band represents a single species, which can be then be identified through DNA sequencing. It is important to emphasize that many of these techniques have been well developed for the medical field and that the application to the more complex matrices in the environmental field will require further research.

2.4 Biodiversity

2.4.1 Recent Trends

The genetic makeup of each individual in a population varies slightly from all other members in the group, and inherently provides a species with the genetic diversity required to adapt, evolve and ultimately survive in a constantly changing environment. A general consensus is that healthy populations of organisms contain a large degree of genetic variability. In the case of humans, approximately 1.4 million single nucleotide polymorphisms have been characterized (SNP, single base pair differences in the genome), the combinations and permutations of which ultimately give rise to the enormous diversity observed in human character traits, abilities, behavior, etc. The subtle differences in genome structure have been exploited by population geneticists to assess population structure, species identification, phylogenetic relatedness, and evolutionary pathways of development.

Although some conventional taxonomy avenues, such as morphological, physiological

and biochemical characteristics, provide a quantitative picture of the similarities and differences among organisms, this approach often gives limited indication to the phylogenetic relationship between different species. Moreover, conventional taxonomy can provide little information about population structure (e.g. degree of outbreeding). Genomics is contributing immensely to research on biodiversity by improving the ability to identify, classify, and determine inter- and intra-species relatedness, ultimately providing a detailed picture of evolutionary selection.

DNA microarrays are likely to play a key role in characterizing genetic diversity. Genotyping microarrays are designed to examine DNA at the sequence level and can be used to detect DNA sequence polymorphisms. DNA fragments unique to different taxonomic groups (e.g., species or population) can be attached onto a DNA microarray and used to rapidly screen samples of DNA extracted from cells for the presence of complementary DNA sequences. An oligonucleotide-based microarray consisting of DNA oligonucleotides of average length (25 to 30 bases) may show significant signal degradation even with a single base mismatch. It is also possible to extend this genotyping approach to DNA sequencing by hybridization. In this case, a complete set of oligonucleotides with all possible combinations of sequence in a given length is synthesized and immobilized onto a microarray. The DNA fragment to be sequenced is broken into small pieces, fluorescently labeled, and hybridized with the immobilized oligonucleotides on the microarray. The sequence of the target DNA can be determined from the pattern of fluorescence bound to the microarray.

Recently, an alternative microarray approach to evaluating genetic diversity has been developed; termed Diversity Array Technology (DArT™) (*21*). DArT™ can provide comprehensive analysis of the genetic diversity in a population without any prior DNA sequence information available for that organism (*21*). Applications of molecular biology tools to assess biodiversity issues have been hampered by the scarcity of genetic sequence information for such a diverse number of experimental organisms. This technology is particularily promising as a low cost, high throughput, molecular tool for genetically characterizing biodiversity of non-model animal species for which little or no genomic sequence data exists.

2.4.2 Research at NWRI

2.4.2.1 Endangered Species Genotyping

DNA microarrays have the potential for use as a forensic tool for detecting plant and animal species and monitoring the trade and transboundary movements of endangered species. For example, DNA fingerprinting methods have recently been shown to be capable of tracing the life of an individual whale from its conception in the North Atlantic to its eventual sale as raw meat in Osaka, Japan.

There is a growing need for new tools to assist with identifying parts or products of endangered species controlled under the Convention on the International Trade of Endangered Species (CITES). Preliminary support has been received from Environment Canada's Office of Enforcement, the Canadian Wildlife Service and the STAGE Program to explore the application of DNA microarrays for using sturgeon DNA sequences to detect the species of origin of caviar products. Environment Canada enforcement officers currently have difficulty identifying the species of origin of caviar products and determining whether fish eggs were obtained from an endangered sturgeon species listed under CITES or from one of the many non-listed sturgeon species from around the world.

Sturgeon mitochondrial DNA (mtDNA) sequences on the cytochrome b gene are being explored as DNA probes to be immobilized on DNA microarrays. At present, the cytochrome b region is assessed through DNA sequencing and while this provides accurate results it may not provide the necessary throughput for a large-scale screening of caviar products. The construction of a "sturgeon chip" could also serve as a model for evaluating the feasibility and cost-effectiveness of developing species-specific microarrays for other environmental genomic applications. This proposal is currently being reviewed as a potential project within the framework of NWRI research.

2.5 Environmental Toxicology

2.5.1 Recent Trends

DNA microarrays have received considerable attention as potentially robust tools for assessing toxic responses induced by drugs and environmental contaminants. This potential application of microarrays has prompted the recent establishment of a National Center of Toxicogenomics (NCT) in the US (http://www.niehs.nih.gov/nct/home.htm). NCT's primary objective is the development and use of DNA microarrays and proteomic tools to address complex issues in the field of human health and environmental toxicology. This new center is a multidisciplinary research center coordinated by the National Institutes of Health (NIH) and the National Institute of Environmental Health Sciences (NIEHS). There are three main areas in environmental toxicology upon which NCT predicts DNA microarrays will have revolutionary impacts:

1. Human risk assessment - understanding biological responses to environmental stressors and identify agents that are a significant risk to human health .
2. Human exposure assessment - improve exposure assessment techniques using microarrays as genetic signatures of exposure.
3. Human susceptibility assessment - identify susceptibility factors that influence an individual's response to environmental agents.

Research is under way to determine whether microarrays can be used as robust indicators of exposure and effects. More specifically, microarrays will be used to determine: a) whether specific toxicants elicit unique genetic signatures and/or tissue-toxic effects, b) whether different animal species show overlapping patterns of gene expression that can be used as models of human exposure, c) whether gene expression patterns can be used to assess the overall health of an organism after exposure to complex mixtures, d) whether low dose chronic gene expression signatures can predict effects of environmental pollutants or toxicants, and e) to characterize biodiversity in a population and account for individual susceptibilities that can mediate disease progression after exposure to a contaminant or toxicant (*22*).

2.5.2 Research at NWRI

Many of the objectives proposed by NCT align with mandates and research initiatives related to the environmental toxicology research program at NWRI. The applications of DNA

microarrays can also be extended into the area of environmental microbiology and effects monitoring, as discussed below.

2.5.2.1 Microbial Toxicogenomics and Environmental Effects Monitoring

A tremendous amount of sequence data will be made available in the near future. Analyses of the newly sequenced microbial genomes has revealed that ~40% of the putative genes identified (predicted open reading frames) encode proteins with unknown functions, indicating that an enormous reservoir of interesting proteins and their biological value remain to be discovered and exploited. The potential applications of new genes and their protein products on a commercial scale has motivated the search for unique biomolecules from the most diverse environments on earth. The nascent but rapidly developing sciences of bioinformatics and functional genomics/proteomics (i.e., methodologies for determining the functions of proteins encoded by unknown genes) will no doubt identify new microbial processes involved in bioremediation and expand our fundamental knowledge of microbial diversity and ecology. Genome sequencing data will also lead to the development of novel technologies and methodologies (i.e., genomic approaches, molecular monitoring tools) for studying the structure and functions of complex microbial communities, including those associated with contaminated environments. The availability of this information will allow considerable expansion of microarrays for environmental monitoring and biodiversity assessment.

In terms of microbial environmental genomics, microarrays have two major areas of application: the simultaneous evaluation of the gene expression profile within a bacterial strain or bacterial community, and the simultaneous detection of a large number of microbial genes. The first approach calls for the extraction of mRNA, and answers obtained in this instance provide information about housekeeping, catabolic or facultatively expressed genes. The second approach relies on the extraction of the microbial DNA, and depending on the genes used, may provide information on the presence or absence of organisms, virulence genes, or the relative abundance of species, genera or microbial domains in a given sample.

In general, microarrays do not offer the low detection levels available by PCR and may not be suited to detection of low abundance organisms in biologically complex samples. A partial answer to this problem lies in the coupling of PCR to microarrays; this may prove quite advantageous, especially when universal primers in conserved sequences such as 16S rRNA genes, DNA gyrases or *cpn60* genes can be used. One would then combine the parallel identification of hundreds of diagnostic sequences simultanously while having to perform only one amplification test and maintain the low detection limits typical of PCR. While the known problems of PCR bias will also manifest themselves in this approach, the possibility of independent confirmation on the same array with different conserved genes and different primers will provide an experimental safeguard.

Great potential lies in using microarray technology to assess changes in the functionality of microbial communities i.e., enrichment or depletion of specific functional groups that are linked to the presence of particular stressors. For example, there are a variety of genes which code for the initial step in alkane degradation (*alkB2* from *Rhodococcus* spp. Q15 and 16531, *alkB* from *Pseudomonas oleovorans*, *alkM* from *Acinetobacter calcoaceticus*) and changes in their abundance and expression would be indicative of both a response to contamination and the potential for natural remediation. Provided that valid uncontaminated reference sites are available, comparisons of the changes in functional gene presence-absence, and in relative

abundance, may be made.

Microarrays may also be used to assess the response of a community to the challenge of a specific stressor or combinations of stressors. One has the option of examining factors such as the total number or range of functional genes, the richness within specific functional genes (e.g., diversity of alkane degradation genes), changes in the proportions of functional genes and/or presence or absence of functional genes. From a regulatory perspective, the development of gene microarrays which target a wide range of functional genes provides a potentially rapid and efficient method of using molecular information to assess changes in biodiversity and community function which, in turn, provides a basis for the determination of environmental effects.

Although, there are major hurdles in the application of microarrays for the analysis of mRNA in environmental samples, they may nevertheless be used to assess gene expression in a community, and responses to external perturbations at the molecular level. Microarrays offer the potential to routinely process large numbers of samples, so the problems of sampling intensity and required replication, due to the heterogeneity in natural systems, can be addressed. The potential exists to produce less equivocal field data allowing much stronger inference regarding causality, and defining acceptable limits for stressors in terms of community response. However, assuming that the challenges may be overcome, traditional toxicology, experimental and field survey studies, separately and in concert, will be required to fully understand the significance of a specific gene array profile and how it reflects community function and the impacts at various trophic levels.

The following is a brief overview of some of the molecular tools that have emerged for analysis of bacterial communities. These approaches may have application for the assessment of changes in biodiversity, monitoring of indicator species and tracking of introduced species. In this context the methods are assessed regarding their potential for environmental effects monitoring. The following approaches are briefly covered: 16s/23srRNA probe techniques, fluorescent *in situ* hybridization or FISH, denaturing gradient gel electrophoresis (DGGE), reverse genome probing (RGP), and DNA microarrays.

Advances in molecular biology have yielded tools such as 16s rRNA probing and sequencing, PCR gene amplification, and hybrids of these methods that can be applied to the analysis of variation and change in microbial communities. These techniques have already been used to index the genetic biodiversity which exists in natural microbial populations, determine phylogenetic relationships, and identify indicator strains from complex systems. Of these new methods, the development of phylogeny-specific molecular probes represents one of the most significant recent advances in determinative microbial ecology. Based on the relatedness of slowly evolving ribosomal RNA sequences, complementary oligonucleotides to these conserved RNA regions permit phylogenetic analysis of organisms obtained from natural systems without the need for cell cultivation. Thus, the identity of community members or indicator organisms which are hard to culture and of unknown ecological significance may now be ascertained, in many cases, to the subspecies level.

Traditional approaches for examining the biodiversity of microbial communities involve culturing the inhabitants. Due to strong environmental stresses imposed during most enrichment and culture procedures (as well as the stringent growth requirements of many microorganisms), the diversity index for most *in situ* environments has been severely underestimated. For example, in a well-studied thermal microbial mat community, 15 unique 16s rRNA sequences were elucidated, and none matched the rRNA sequences of organisms cultivated during previous

studies. The data suggest that a large number of non-culturable organisms were present in the original sample.

Methods for using phylogenetic probes have undergone steady refinement in terms of sensitivity towards target organisms, decreased background interference, and procedural refinements for *in situ* application in more complex systems. In a study examining antibiotic-mediated fluctuations in the microflora of the bovine rumen, it has been shown that cultural enumeration techniques were less sensitive to changes in the relative abundance of organisms than were fluorescent rRNA probe conjugates. Other studies using fluor-conjugated phylogenetic probes have demonstrated single-mismatch specificity between target and non-target sequences and have also proven valuable for examining the composition of a variety of natural communities.

Denaturing gradient gel electrophoresis (DGGE) analysis is another molecular approach applicable to community-level characterization of microbial populations. Based on the electrophoretic separation of PCR-amplified 16s rRNA gene fragments of the same length (PCR-amplified rDNA), DGGE may be used to profile microbial nucleic acids obtained from a range of environments both qualitatively and semi-quantitatively. Examination of *in situ* microbial communities from microbial mats and aerobic biofilms, demonstrated that from between 5 to 10 different bands could be detected for each population, with some bands shared between different populations. The sensitivity of this approach has been determined using mixed cultures of known composition. Studies have indicated that individuals accounting for less than 1% of the total community (*Desulfovibrio* spp.) can be reliably detected. This approach was also used in combination with hybridization techniques to demonstrate the occurrence of sulfate-reducing bacteria within a biofilm cultivated aerobically, demonstrating that anaerobic microniches formed as a result of metabolic oxygen consumption thereby creating suitable habitats for anaerobes.

Reverse sample genome probing (RSGP) may be used to determine the presence of indicator strains within environmental samples or microbial communities. This is done by denaturing bacterial DNA obtained from target organisms (the standards) and spotting the DNA onto a master filter. Environmental DNA is then labeled and hybridized with the filter to identify which of the bacterial genomes present on the filter is present in the environmental sample. The standards used during this procedure are available commercially or can be enriched and isolated from *in situ* environments. A study of 56 Alberta oil field sites demonstrated the potential for identifying constituent SRB (sulfate-reducing bacteria), using 35 different standards of SRBs which exhibited little or no cross-reactivity. Examination of these sites using genome probes demonstrated that the salt concentration of the water samples defined the SRB community, with two distinct SRB populations being evident. From the 35 SRB bacterial genomes utilized as standards, 10 were unique to freshwater, 18 to saline waters, and 6 to both. In addition to providing qualitative data, this method may also be used quantitatively (provided that the cultivation step is not performed) by using scintillation counting to determine the amount of bound radioactivity for each specific standard genome. While this technique does not normally establish the importance of community members which have not previously been isolated, it does permit the diagnostic detection of indicator or target organisms.

2.5.2.1.1 *Current Projects at NWRI in Microbial Toxicogenomics and Environmental Effects Monitoring*

1. Development of a DNA-microarray for Environmental Effects Monitoring. In conjunction with collaborators at NRC-BRI, Montreal, Dr. John Lawrence is engaged in the development of an Eco-microarray system to allow screening for the presence, abundance and diversity of a suite of biogeochemical and degradative genes that are critical to ecosystem function. An initial array design has been achieved with successful hybridization with DNA extracted from hydrocarbon affected and unaffected sites.
2. Monitoring the fate and effects of GMOs using GFP. A GMO surrogate, *Pseudomonas putida* modified to constitutively express GFP was released into a contained model river system, and its abundance tracked using a combination of an antibiotic resistance marker, GFP and 16S rRNA probing.
3. Applications of DGGE and FISH to assess community structure.Studies have been completed on monitoring a degradative microbial community, and deep subsurface microbial communities. Further studies were carried out using an array of rRNA probes to assess biodiversity in river-biofilms under different treatment regimes. Projects are in progress using these techniques to assess the impact of nutrients and an array of contaminants on microbial community structure. Ultimately this will be coupled to DNA microarray assessments.

2.5.2.2 Fish Toxicogenomics and Environmental Health Assessment

The biggest challenge to assessing the environmental impact of a contaminant is to determine the relative health of an organism in an impacted environment. Two general biological approaches to health assessment have been used to characterize environmentally impacted sites: exposure and effects assessment. Biomarkers are often used as a measure of exposure to a contaminant. However, it is often difficult to conclude health effects based on biomarkers. For example, sporadic contamination by a xenobiotic may induce certain biological responses but not affect long term health of the organism. Alternatively, effects monitoring examines alterations and perturbations in biological homeostasis of an organism. Effects monitoring is limited to the context of the effect being examined; a lack of effect does not necessarily imply a healthy population. Often times overt manifestations of health indices (e.g., gross morphological effects) are required for effects to be concluded. As such, effects monitoring has limited ability to predict and prevent environmental impacts by contaminants. Overt effects can serve as a trigger for remedial action to clean or rehabilitate contaminated areas. In addition, both exposure and effects are difficult to assess in areas where sporadic release or low level chronic exposures are occurring.

DNA microarrays provide the potential to bridge the limitations imposed by exposure and effects monitoring. Upregulation or downregulation of genes (or families of genes) may be indicative of exposure to a contaminant or class of contaminants (i.e., biomarker). Alternatively, up or down regulation of groups of genes within a particular pathway may be used to predict the effect of the exposure. In most cases a multitude of gene expression patterns may be altered in response to chemical exposure (i.e., polygenic). Assessing polygenic interactions is essential, since phenotypic alterations (i.e. effects) induced by contaminant-induced injury are never the result of a single gene being affected (*23*). Thus, microarrays may be important in predicting which effect to look for in a contaminated site.

Ultimately, applications to environmental toxicology will depend on basic research that will validate microarray technology against traditional methods of assessing exposure and effects. A fundamental question is whether patterns of gene expression correlate with exposure

to known toxicants (e.g., one chemical-one signature), and whether patterns of gene expression can be used to assess the mechanism of toxicity and subsequently the effect on organism health. In an ideal situation, contaminant-induced gene expression would elicit patterns of expression limited to a biological response or a biochemical pathway relevant to the mechanism of toxicity (*24*). However, to date relatively few correlations between gene expression and toxic stress can be directly linked to mechanisms (*24*). This is because contaminant-induced gene expression may manifest itself in several ways in a biological system, depending on the mode of action of the contaminant, dose, contact time, and route of exposure. For example, contaminants that interfere or bind to intracellular signaling proteins (i.e., kinases) may affect a multitude of biochemical pathways, leading to a diverse manifestation in the expression of relatively unrelated genes or gene families. Other types of contaminant exposure may induce gene expression profiles more restrictive to specific biochemical pathways (i.e. PAHs and CYP family genes) (*25-27*). Recent data suggests that relatively simple and targeted microarrays can be used to assess exposure to major groups of environmental contaminants (i.e. heavy metals, PAHs, etc.)(*25-27*). More comprehensive arrays examining global expression profiles (i.e., all genes) may provide powerful tools for resolving subtle gene expression differences between closely related chemicals (i.e., increased resolution for discriminating individual chemical signatures).

In light of future research initiatives, it has been suggested that the ultimate goal in toxicology is to match responses of individuals to different environmental stimuli (*28;29*); an approach currently being investigated in the field of pharmacogenomics to determine individual drug response within a human population. Efforts are underway to comprehensively genotype human populations based on the diversity of single nucleotide polymorphisms (SNP) present in the human genome. The result of these efforts will dramatically facilitate drug-development, since individual responses can be correlated and mapped to genotypic profiles. A large-scale investment by pharmaceutical companies into identifying and patenting SNP sequences is underway (*30*).

This pharmacogenomic approach can have significant implications for environmental toxicology; the ability to predict the outcome that environmental stressors may have on population structures. It has been demonstrated that isolates of the same species can display differential susceptibilities to environmental contaminants (*31-33*), suggesting that contaminant exposure may dramatically alter intraspecific natural selection processes and consequently the evolutionary selection of individuals within a population. Determining which individuals or populations are susceptible to environmental contaminants may be used as predictive measures of contaminant-induced evolutionary selection. This information will be instrumental in conserving and protecting sensitive populations, and may be used to ultimately assess evolutionary impacts of environmental stressors on species composition and sustainability.

2.5.2.2.1 *Projects at NWRI in Toxicogenomics and Environmental Health Assessment*

Currently at NWRI, there is a research project being developed for validating DNA microarray approaches with conventional exposure and effects assays. This research is aimed at examining the application of DNA chips in monitoring exposure and assessing physiological effects in rainbow trout exposed to heavy metals. The project is currently in review at NSERC under the Strategic Grants in Environmental Research. A DNA microchip has been developed in rainbow trout (collaborators at University of Waterloo and Victoria) that consists of

approximately 150 genes. Many of the genes represented on the chip are of interest to scientist at NWRI, including thyroid-related, MFO, immune, endocrine and reproductive genes. The scope of the project involves the characterization of cellular responses to heavy metal exposure, and subsequently assessing the applicability of the technology to predict exposures and effects in laboratory and caged fish field trials.

2.6 Analytical Chemistry

2.6.1 Recent Trends

The need for automation, miniaturization, and high throughput analysis in many molecular applications has inspired innovative product development in the physical, chemical, and engineering sciences. Recent demands have spawned the development of miniaturized prototypical 'labs-on-a chip', many of which have been developed in response to the growing applications of genomic technologies. Recent advances include the development of an integrated chip device capable of extracting nucleic acids, running PCR reactions, fluorescent labeling of nucleic acids, and hybridizing samples to microarrays in a compact lab-on-a-chip device no bigger than a credit card (*34*). Miniaturization and automation to this scale allows for rapid, high throughput, routine, and ease of use sample analysis.

Integrated lab-on-a-chip devices have now moved beyond basic genomic applications and into other biological and chemical applications. Labs-on-a-chip have been developed for capillary electrophoresis used in the purification and identification of biomolecules (Agilent Technologies). Prototype lab-on-a-chips have also been developed to characterize enzymatic reactions (*35*), identify organophosphate pesticides in natural waters (*36*), and for general HPLC applications (μChemLab™, Sandia Laboratories). Sandia Laboratories has recently developed a prototype hand-held, disposable, lab-on-a-chip HPLC system (μChemLab™) capable of rapidly (within minutes) identifying environmental chemicals at concentrations as low as 10 -100 ppb. The integrated chip device is small enough to fit into a pea pod. Siemens has also released a prototype lab-on-a-chip (CENSAR) for applications in water quality testing (pH, dissolved oxygen, conductivity, redox, temperature, and free chlorine). The CENSAR chip device measures 25 mm^2. Microchip capillary electrophoresis systems have also been recently developed for detecting organophosphate pesticides, and have been demonstrated to have potential utility for on site field testing applications for detecting pesticides such as paraoxon, methyl parathion, and fenitrothion in natural river water samples (*36*). These miniaturized devices have several advantages over conventional analytical chemistry technology including portability, size, cost efficiency (chips are relatively inexpensive to manufacture once molds are designed), rapid sample identification, and micro-volume analysis.

2.6.2 Research at NWRI

DNA microarray technology could find potential utility in NWRI programs related to toxicity identification and evaluation (TIE) of complex chemical mixtures, and in analyses performed by NLET (National Laboratory for Environmental Testing). Currently, TIE analysis requires multifaceted biological testing of fractionated samples in conventional bioassays. Microarrays offer the potential utility of rapidly screening fractions for biological effects, without prior knowledge of the contaminant or its biological action. Distinct patterns of gene expression may also be used to 'fingerprint' a specific chemical or group of chemicals. Upregulation of genes targeted to specific biochemical pathways may be indicative of mechanisms of action, and subsequently predictive of potential effects to an organism. Predicted effects can be cross validated using targeted conventional bioassays.

3. BUILDING GENOMICS CAPACITY AT NWRI

3.1 Approach

It is essential that NWRI scientists develop 'hands-on' expertise. Without expertise in genomics research applications, scientists cannot properly evaluate grant proposals, review research papers, advise the department on genomics related issues or do research. Lack of expertise will also affect the ability of research scientists to critically evaluate scientific findings required for regulating GMO development and release (as required under the new CEPA regulations).

Although other options were discussed, the NWRI Molecular Biology Planning Group felt that an 'in-house' genomics research facility was the most suitable option. This conclusion was based on the following rationale:

1. Biotechnological development is a major focus in many university research programs, driving the development and release of patented or marketable products. Due to the 'high-tech' nature of genomics research, NWRI may encounter legal issues related to patents and unrestricted use or access to the products developed in collaboration with external research agencies. This has already become an issue with some researchers at NWRI who recruit external agencies for genomics-based research support.
2. An in-house genomics facility would allow scientists to explore research avenues directly applicable to NWRI research mandates, without being dependent on securing collaborations with university researchers. Although these techniques or procedures may be available within university research settings, they may not be readily accessible by NWRI research scientists.
3. In-house development of molecular techniques or products will permit unrestricted use of these commodities in other relevant areas of research without relying on permission of accessibility rights to be granted.
4. Researchers at NWRI are encouraged to collaborate with university partners for product or technical development, but should be made aware of legal rights to market advanced techniques in this rapidly changing field. Independent product and research development at NWRI will circumvent these legal challenges.

Group members agreed that a central *user-based* facility should be developed at NWRI to allow NWRI scientists unrestricted access to equipment and facilities used for genomics research. The advantage of having a central user-based facility is that individual equipment needs are not duplicated, facilitating an economically feasible transition into developing genomics capacity at NWRI, while permitting the acquisition of state-of-the-art equipment.

Developing a state-of-the-art laboratory would also allow research scientists to exploit a diverse array of cutting-edge molecular techniques to be employed in their research program (Table 3). The laboratory should be implemented with the equipment listed in Table 4.

The disadvantage to this user-based approach, is that NWRI scientists would be required to develop their own genomics-based portion of their research programs. It was generally acknowledged that this aspect of biology is lacking at NWRI. Nevertheless, this disadvantage is countered by the inherent requirement for individual scientists to become familiar with molecular techniques and to develop a critical awareness and expertise in exploiting these molecular research tools. Staffing of a user-based facility by individuals familiar with molecular tools will facilitate integration of genomics-related research into existing research programs (see below). However, it is not imperative that each researcher develop genomics capacity in their individual programs.

Table 3. Priority molecular biology tools for NWRI and their applicability to Environment Canada research programs.

Molecular Technique	NWRI Research Application
DNA and protein microarrays	GMOs, Bioremediation, Environmental Toxicology, Biodiversity, Pathogens
Denaturing Gradient Gel Electrophoresis (DGGE)	Bioremediation, Biodiversity, Environmental Toxicology
Fluorescent In Situ Hybridization (FISH)	Environmental Toxicology, Bioremediation, Pathogens
Polymerase Chain Reaction	GMOs, Bioremediation, Environmental Toxicology, Biodiversity, Pathogens
Differential Display	Environmental Toxicology
Restriction Fragment Length Polymorphism (RFLP) / Amplified Fragment Length Polymorphism (AFLP)	Biodiversity, GMOs
Microsatellite Analysis	GMOs, Bioremediation, Environmental Toxicology, Biodiversity, Pathogens
DNA sequencing	GMOs, Bioremediation, Environmental Toxicology, Biodiversity, Pathogens
Gene cloning and recombinant DNA transfer	GMOs, Bioremediation, Environmental Toxicology, Biodiversity, Pathogens

Table 4. Equipment needed to develop a user-based genomics facility at CCIW.

Equipment	Quantity	Estimated Cost
Robotics microarrayer	1	$140,000
Microarray scanner	2	$180,000
Multi-block PCR system	1	$30,000
Biosafety cabinets (Level II)	2	$30,000
Freezers (-86°C)	2	$30,000
Ultra centrifuge	1	$100,000
Refrigerators (4°C)	2	$2000
Electrophoresis and associated DNA/RNA analytical equipment	various	$30,000
Freeze dryer	1	in house
Film developer	1	$30,000
TOTAL (Immediate needs)		**$572,000.**
DNA Sequencer (2002)		$250,000
TOTAL		**$822,000.**

The user based system would be overseen by a committee that would facilitate the research needs and requirements of the facility. One recommendation was that the existing Molecular Biology Planning Group participants act as the overseeing committee.

3.2 Staffing Requirements

Staffing for a user-based genomics facility would require technical specialists to maintain and run the specialized equipment, and for assisting NWRI scientists in their efforts to integrate genomics-based research into existing research programs. Future staffing positions are outlined in Table 5.

3.2.1 Immediate Staffing Requirements

A senior level technician, or junior professional, would be required to maintain the facility, and operate specialized equipment (i.e. robotics arrayer and DNA sequencer). This person should be well versed in the use of molecular techniques, and familiar with the latest developments and applications in molecular biology. This person could be used as a resource and consultant for NWRI scientists who wish to pursue genomics-based research in their programs.

However, the technician would not be used to develop genomics components for individual researchers .

It was also recommended that a bioinformatics specialist be hired to help scientists design experiments, analyze data, and interpret results. This person could also be responsible for web-development of network databases centered, maintained, and developed at NWRI. Such development of online databases is in keeping with other Federal Government and NWRI initiatives such as CISTI (Canadian Institute for Science and Technology Information) and CWRN (Canadian Water Resource Network). Together with the senior technician, this individual will also be responsible for maintaining and organizing the facility.

3.2.2 Future Staffing Requirements

A future research position in genetic biodiversity should be considered. Research expertise in this area is non-existent at NWRI. Genomics applications are extensively used as robust tools for assessing biodiversity issues, and will significantly complement current conventional approaches to ascertaining biodiversity.

Table 5. Research personnel required for genomics facility

Position	Qualification	Responsibility
Molecular Biology Technician or Junior Professional	MSc. (minimum) (Specialization in Molecular Biology)	• Equipment maintenance and routine operation of genomics-related equipment. • Act as a consultant for NWRI scientists for integration of genomics-based research into existing research programs.
Bioinformatics Specialist	MSc. (minimum) (Specialization in Bioinformatics)	• Assist in experiment design, data acquisition and analysis. • Generation of web-based genomic databases and analysis packages. • Act as a consultant for NWRI scientists for integration of genomics-based research into existing research programs.
Research Scientist	Ph.D (Specialization in Molecular Biology)	• Develop a research program based on genetic biodiversity or species at risk.

4. SUMMARY

Advances in the field of genetics will significantly impact existing research programs at NWRI. Progress towards developing expertise in this area will lay the foundation for research challenges that NWRI will inevitably face in the future. NWRI's international recognition will be enhanced by participation in the development of these cutting-edge genetic tools, and using them to complement the high quality research done at the institute. It is inevitable that highly advanced molecular tools will become standard laboratory assays in many areas of ecosystem health research, and that moving towards this area establishes a presence and expertise for NWRI. Failure to pursue these tools may severely affect the overall impact of our scientific contribution in many areas of environmental research. Incorporating genetics-based research into existing programs will support responsible decision-making policies, and help Environment Canada meet its mandates to maintain a clean and healthy environment.

5. GLOSSARY

AFLP - amplified fragment length polymorphism. A technique by which DNA is 'profiled' based on the ability of specific or random primers to amplify DNA cleaved by restriction enzymes. AFLP is often used as a measure of biodiversity.

DGGE - denaturing gradient gel electrophoresis. A technique in which DNA fragments of similar length can be distinguished based on their melting temperatures. DGGE is often used to identify distinct species.

differential display - a molecular technique used to profile mRNA for gene expression analysis.

FISH - Fluorescent *in situ* hybridization. *In situ* assay using fluorescently labeled nucleotides, designed as complementary sequences to a target gene or sequence. Used to identify individual cells or organisms in a complex assortment.

gene stacking - inclusion of multiple traits into the genome of a recipient host.

introgression - hereditary trait within a population arising from stable transfection and integration of foreign gene(s) into the genome of a recipient organism.

microsatellites - genomic DNA characterized by short (2-5 basepair) tandem repeats with variations in copy number among individuals. These DNA sequences tend to be randomly distributed throughout the genome and are subject to replication slippage that leads to length variation. Microsatellites are often used to type individuals in a population.

PCR - polymerase chain reaction. *In vitro* amplification of DNA

RAPD - random amplified polymorphic DNA. PCR amplification of DNA based on random primer annealing. RAPD is often used as a measure of genetic diversity.

RFLP - restriction fragment length polymorphism. A technique by which DNA is 'profiled' based on the ability of a restriction enzyme(s) to cut DNA. RFLP is often used as a measure of biodiversity.

transfection - incorporation of foreign gene into a recipient organism.

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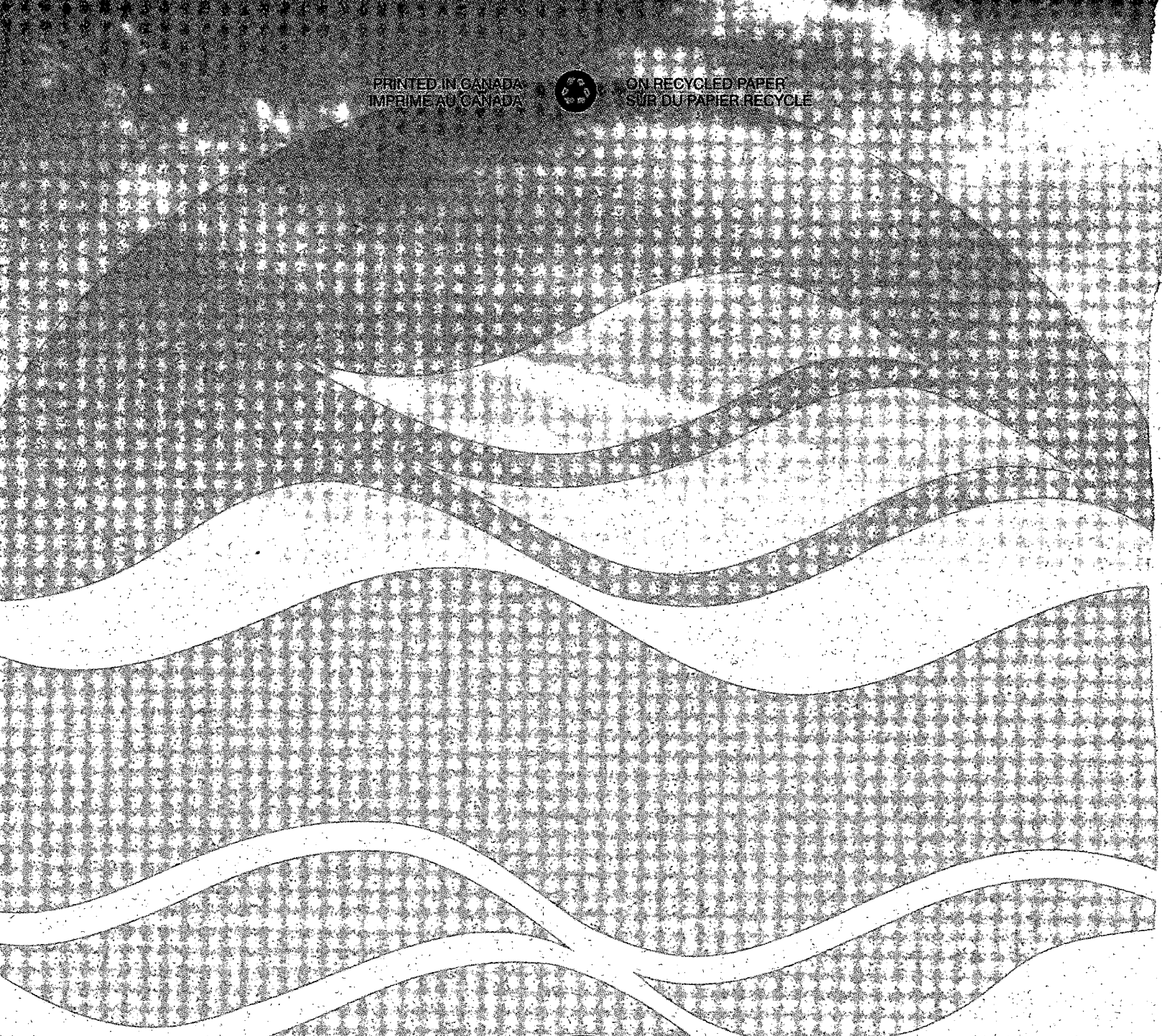

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