Star Grant CR-83075701:
Children' Vulnerability to Toxic Substances in the Environment

Analysis of Genotoxic Biomarkers in Children Associated with a Pediatric Cancer Cluster and Exposure to Two Superfund Sites

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Chicago IL

RESEARCH PROPOSAL

Objective

To Evaluate the utility of specific biomarkers for assessing cancer risk in a pediatric population following environmental exposure to genotoxic chemicals

- Measure biomarkers of effect in children from an exposed population with a documented increase in childhood cancer incidence and compare them to measurements in unexposed children.

RESEARCH PROPOSAL

Specific Aims

1. To utilize the *HPRT* biomarker system to investigate the genetic effects of exposure by determining the frequency and mutational spectra in a high risk pediatric cancer population from Tom's River N.J. who were exposed to contaminated drinking water from two Superfund sites.

2. To compare the frequency of chromosomal aberrations (CA) as a biomarker of effect from exposed and unexposed children to investigate prevalence of genomic damage

3. To determine genotype frequencies for 18 polymorphisms of 11 metabolic genes as biomarkers of susceptibility in exposed children with cancer, their siblings and unexposed children.

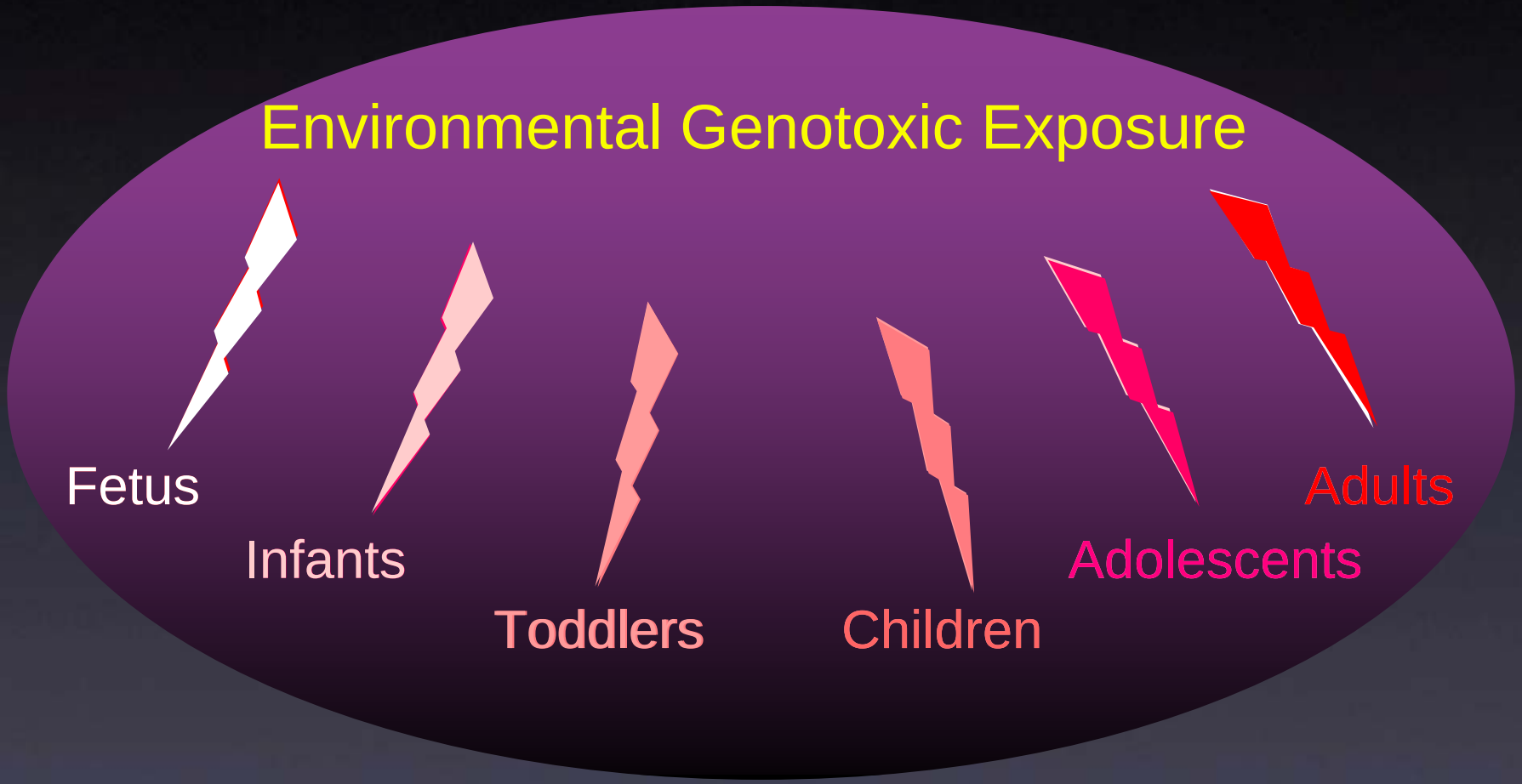
Current Status of Biomarkers for Assessing Cancer Risk

- Somatic mutations are promising biomarkers for cancer risk because they
  can capture genetic events that are associated with malignant transformation. To date there are no studies to support a relationship between frequency or specta of somatic mutations and cancer risk.

- The association between chromosomal aberrations and neoplastic transformation has been well established. Two major cohort studies performed in the Nordic countries and Italy have demonstrated that the frequency of CA's in peripheral MNCs is predictive of cancer risk.

- Carcinogenic chemicals can be directly mutagenic or require metabolic activation
  to gain genotoxic potential. Metabolic enzymes also exist that detoxify carcinogens *in vivo.* There is growing evidence that specific polymorphisms in
  these genes are associated with cancer risk

Genetic Effects of Genotoxic Exposure in Humans



Susceptibility of Environmental Exposure in Children

Exposure

- Food Consumption
  - Children consume 7 times as much water per pound than an adult during their first 6 months of life.
  - Children eat 3 -4 times more food per pound as an adult during there first 5 years of life.

- Behavior
  - Unable to remove themselves from noxious environments
  - Oral exploration

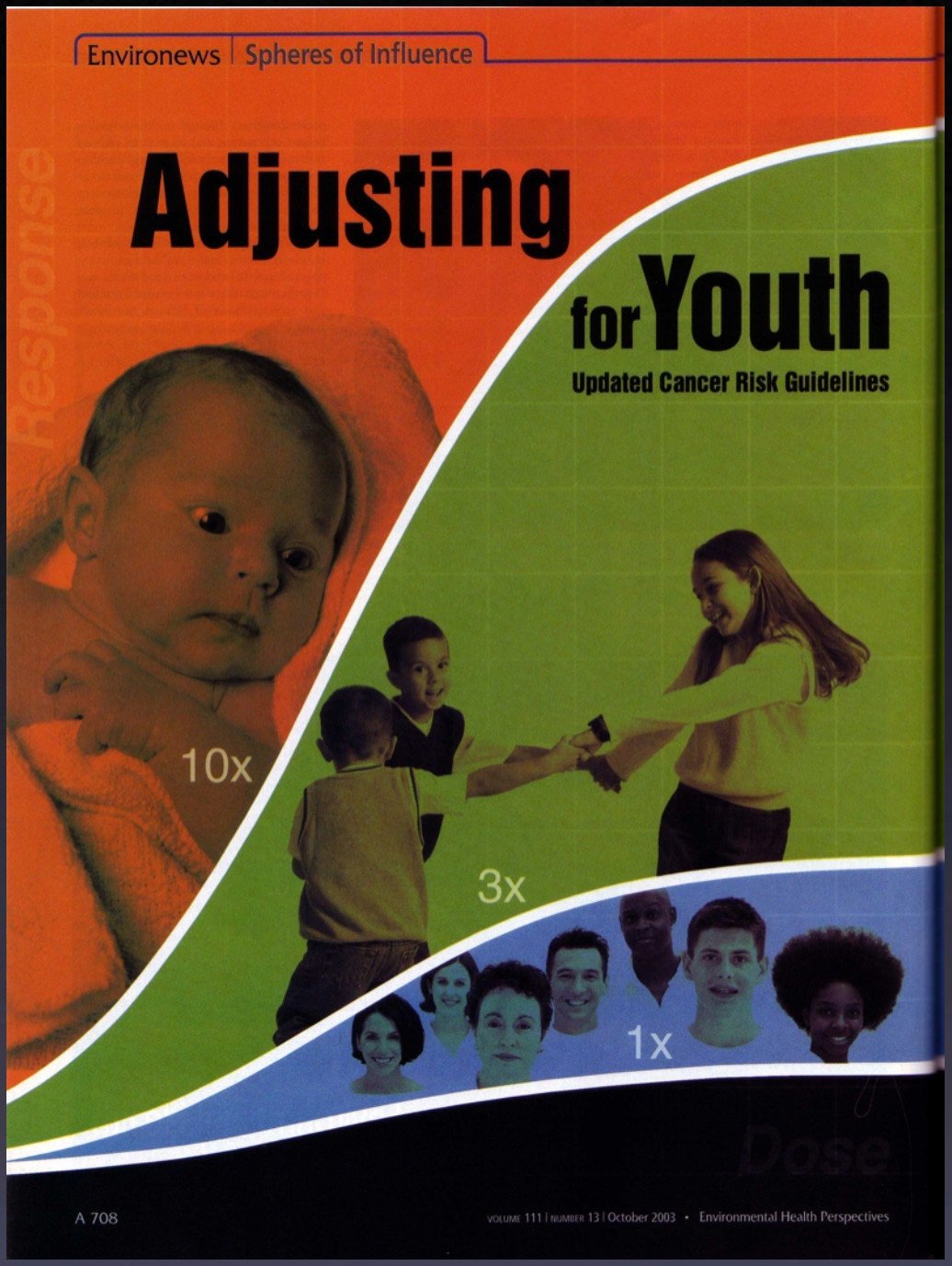
Susceptibility of Environmental Exposure in Children

Exposure

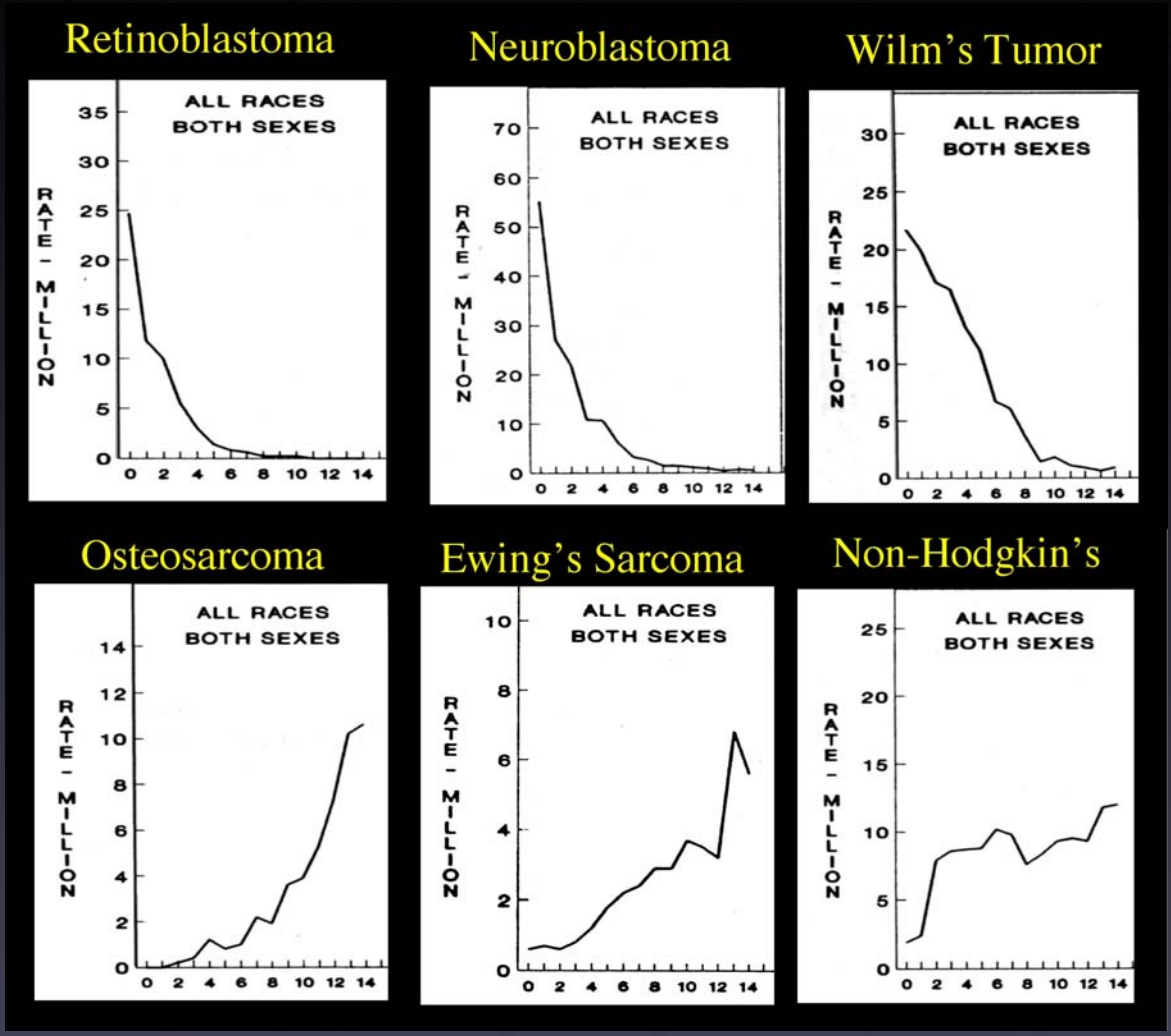
- Physical Location
  - Developmentally dependent

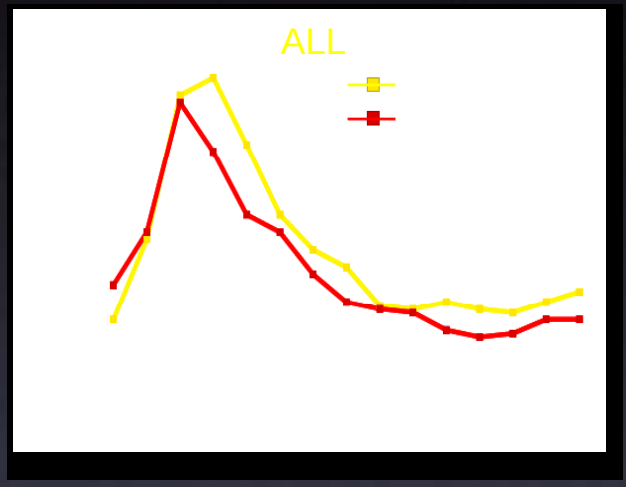
- Breathing Zones
  - Lower breathing zones that accumulate higher concentrations of heavier chemicals.

- Oxygen Consumption
  - Higher surface to volume ratio resulting in an O_2 consumption 2 fold higher than adults.

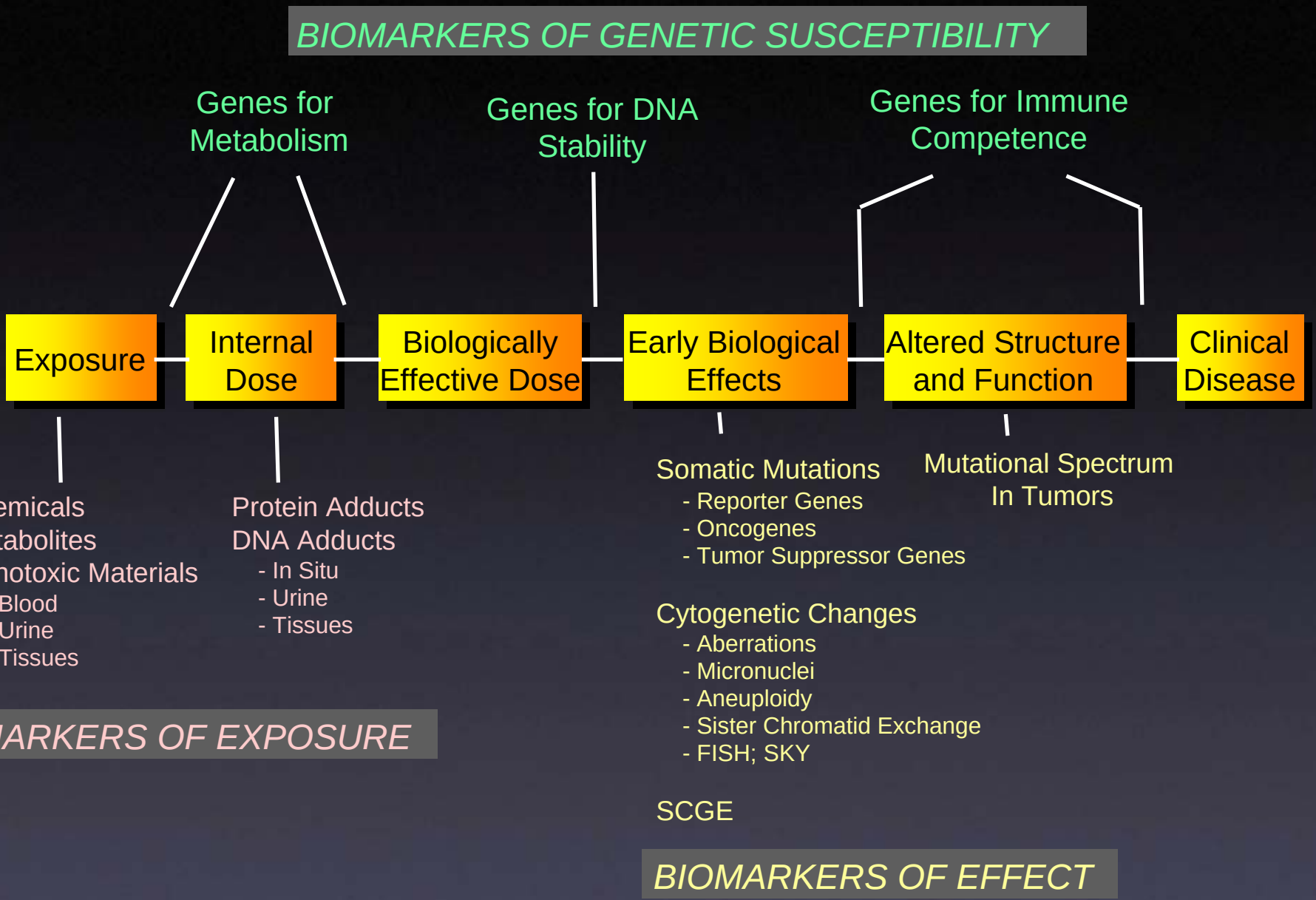

Environews | Spheres of Influence
Adjusting
for Youth
Updated Cancer Risk Guidelines
Response
10x
3x
1x
Dose
A 708
VOLUME 111 | NUMBER 13 | October 2003 • Environmental Health Perspectives

Age Specific Incidence of Cancer





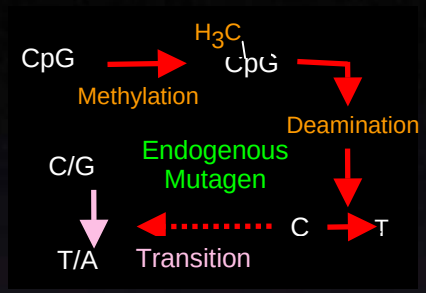
Biomarker Monitoring and Assessment



Research Program

Genotoxic Exposures

Fetus



Young adult

Infant

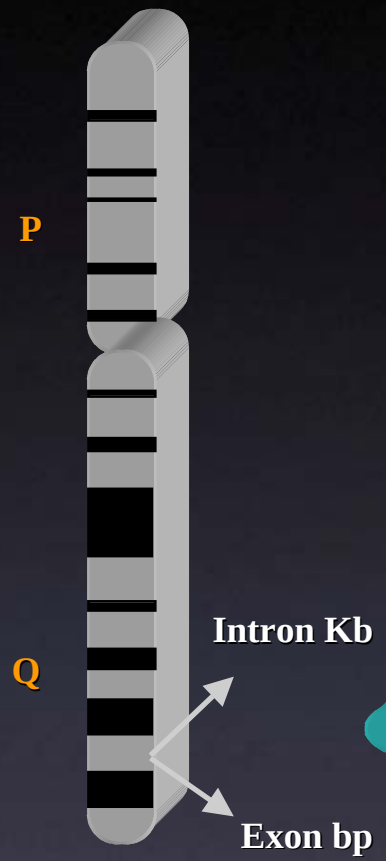
Pre-pubertal

Toddler

Somatic Mutations

Gene Model

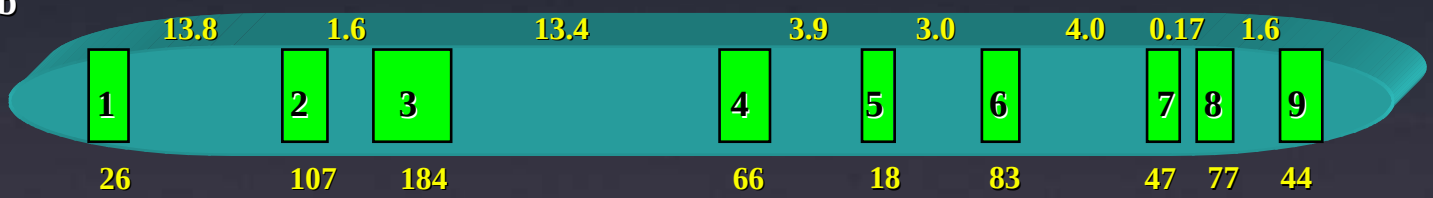
X Chromosome



Clinical Diseases Associated with Germinal Mutations at the *HPRT* Locus

- **Lesch - Nyhan Syndrome**
- **Gout**

***HPRT* Gene at Xq26**



Non-malignant peripheral T cells

The *HPRT* Biomarker System

HPRT is a non-essential gene involved in purine salvage

HPRT gene is located on the X-chromosome at Xq26

– Therefore this locus is functionally heterozygous in which a single mutation can result in mutant phenotype.

– Mutations in this gene can be used as a biomarker of effect, reflective of genome wide mutational events.

Selection of *HPRT* mutant clones in peripheral T-cells

– Lymphocytes that have a mutation resulting in altered *HPRT* activity will grow in the presence of the purine analog 6-thioguanine.

– This allows for the selection of rare mutational events.

The *HPRT* Biomarker System

HPRT mutations do not result in altered cell function

– This allows for an accurate determination of somatic mutant frequency.

Molecular Analysis

– Mutant isolates can be expanded in vitro.

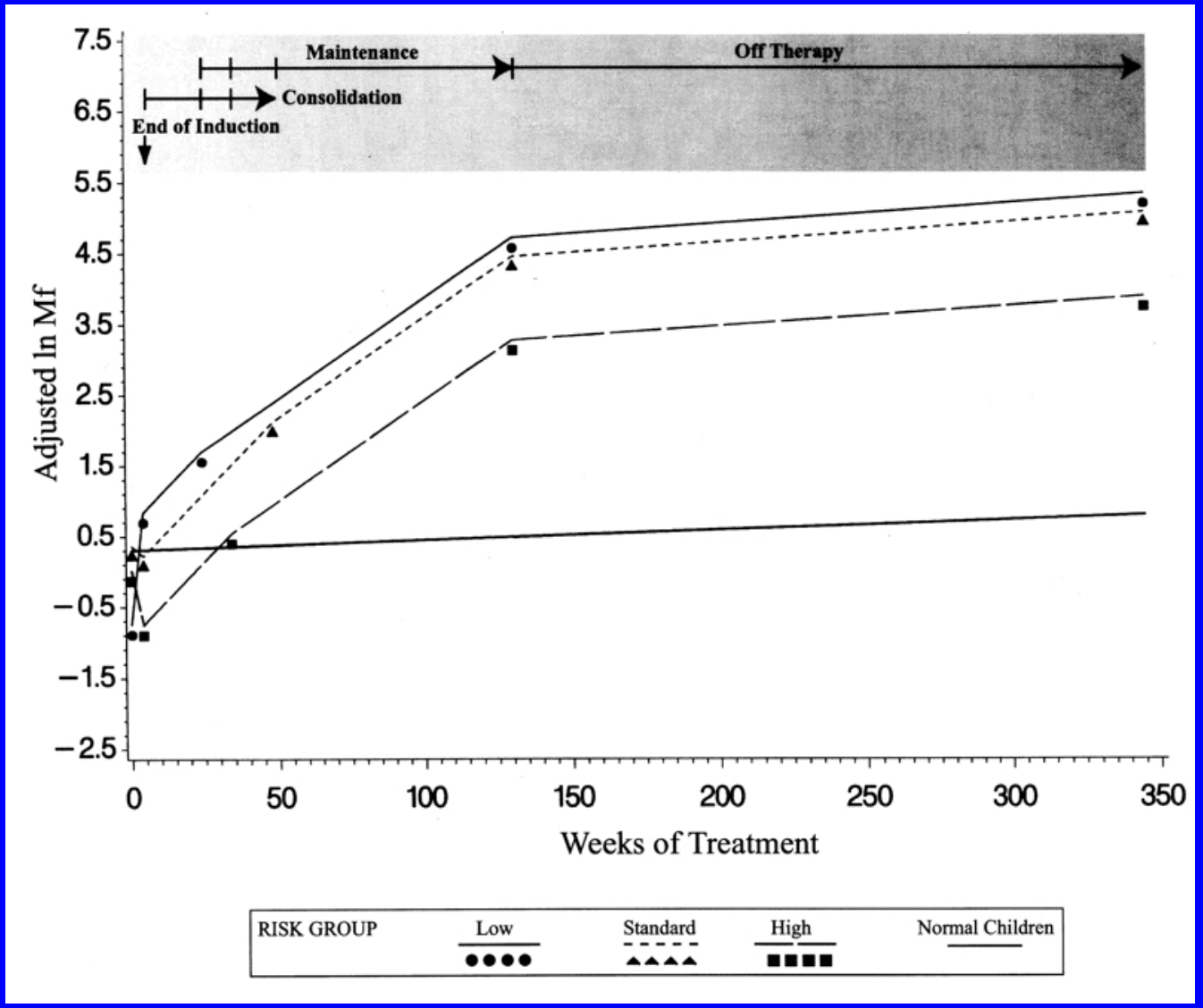
– *HPRT* mutant clones can be characterized at the molecular level to gain insight into mutational mechanisms and mutational spectra.

Determination of T cell clonality

– *HPRT* mutants can be specifically identified by their T cell receptor (TCR) CDR3 region.

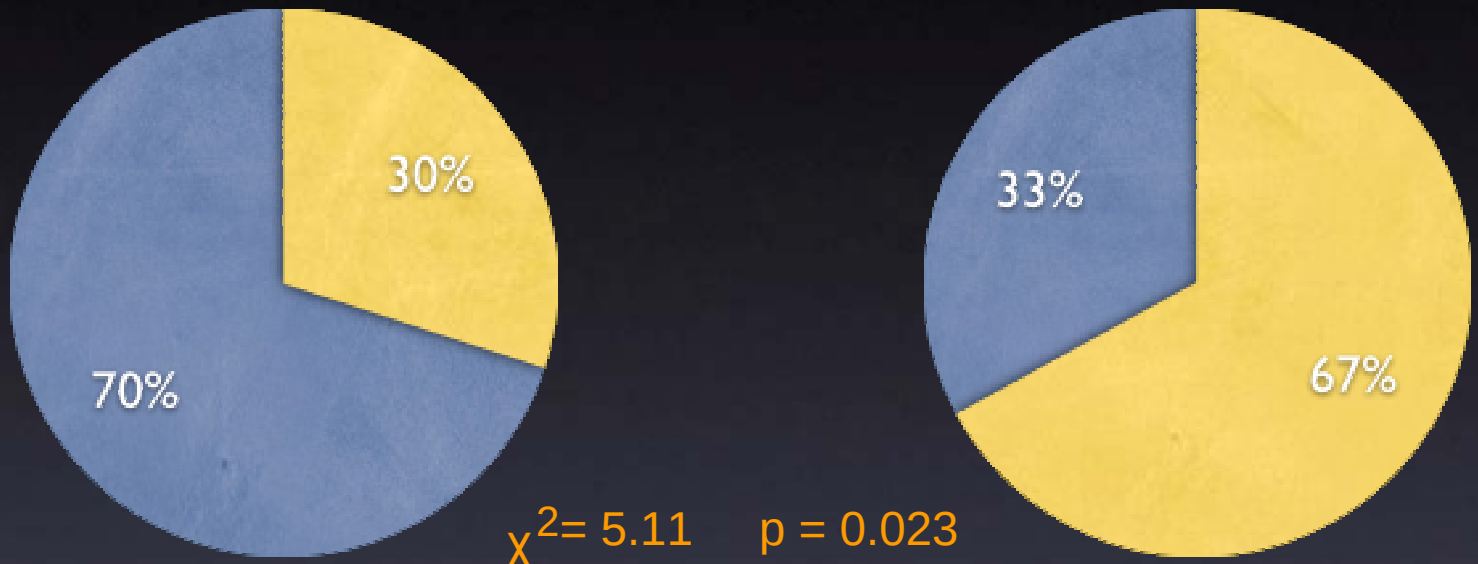
– Allows for the identification of in vivo sequential mutational events in independent single mutant isolates or those associated with a proliferating clonal population.

Longitudinal Genotoxic Effects of Treatment for ALL



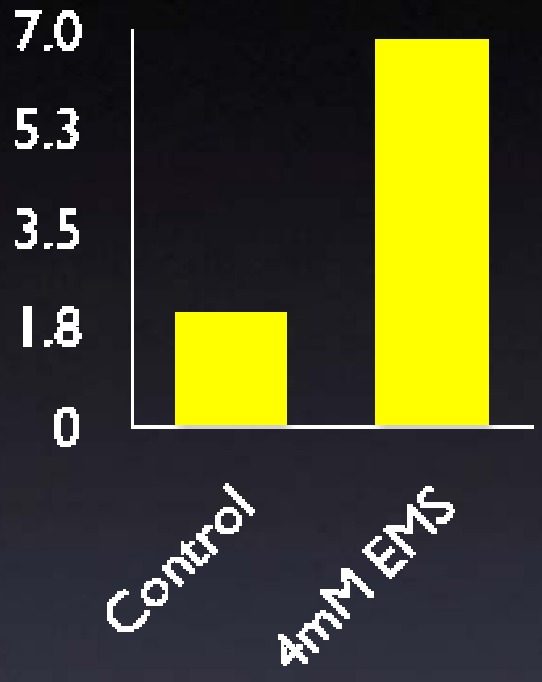
Rice et al. 2004. Cancer Research

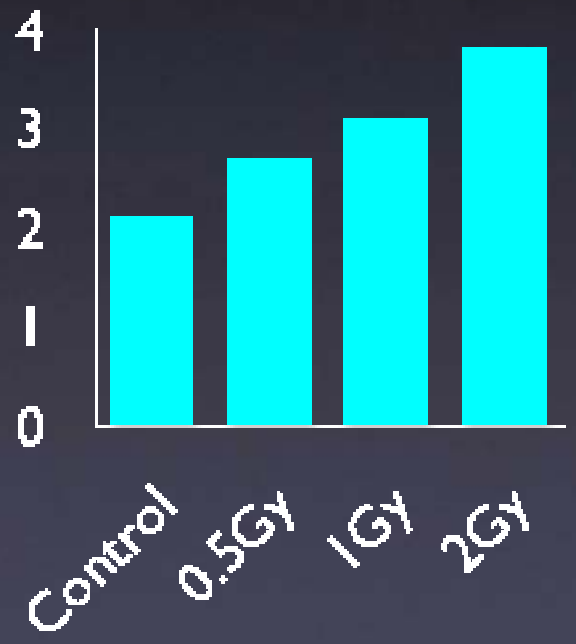
Distribution of Aberrant V(D)J Recombinase mediated *HPRT* Deletions in Newborns Following Transplacental Exposure to Environmental Tobacco Smoke

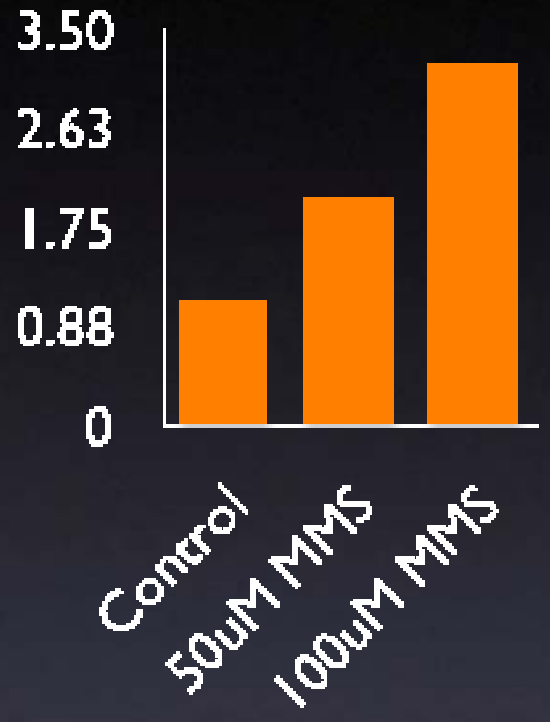


Finette et al, 1998. Nature Medicine

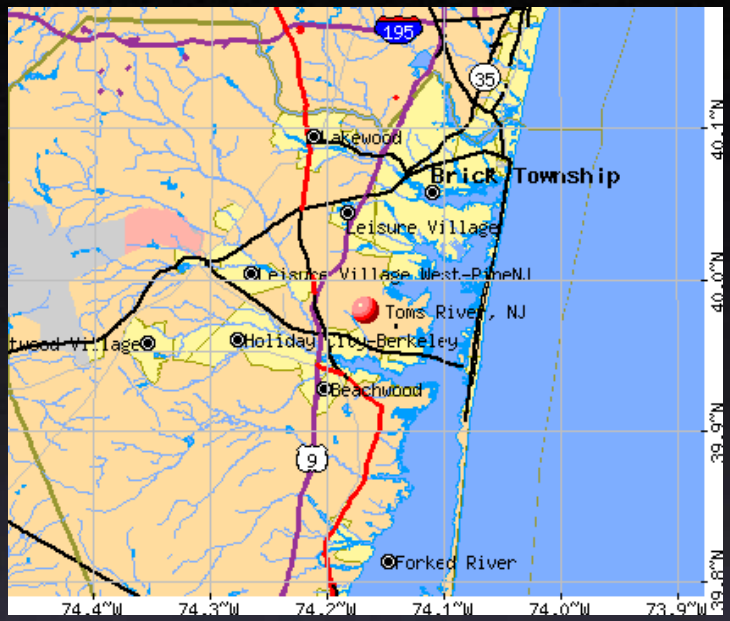
Frequency of *in vitro* V(D)J recombinase events following exposure to alkylating agents (EMS and MMS) and radiation







Dover Township resides in Ocean County N.J. off the Atlantic seacoast and was established in 1767. Within Dover township resides Toms River Village



Background for Tom's River N.J. Pediatric Cancer Cluste

Children residing in Dover Township, New Jersey between 1979-1995 are an ideal population for evaluating biomarkers of the health effects of exposure because their elevated risk of childhood cancer is well-documented and has been linked to their exposure to industrially contaminated groundwater.

Extensive epidemiological studies by the New Jersey Department of Health and Senior Services and the U.S. Agency for Toxic Substances and Disease Registry found that the incidence of childhood cancer in Dover Township during 1979-1995 was 34% higher than the statewide rate.

Background for Tom's River N.J. Pediatric Cancer Cluste

In the Toms River, a section of Dover Township defined by four census tracks, the incidence was 70% higher than the statewide rate. In particular female children with ALL under 5 years of age, as well as brain and other CNS tumors. A case-control study indicated that the cancers were associated with exposure to contaminated drinking water, either *in utero* or during early childhood and extensive toxicologic and hydro-geologic studies demonstrated that chemicals and radionucleotides have contaminated public groundwater in some areas of Dover Township for the past 50 years.

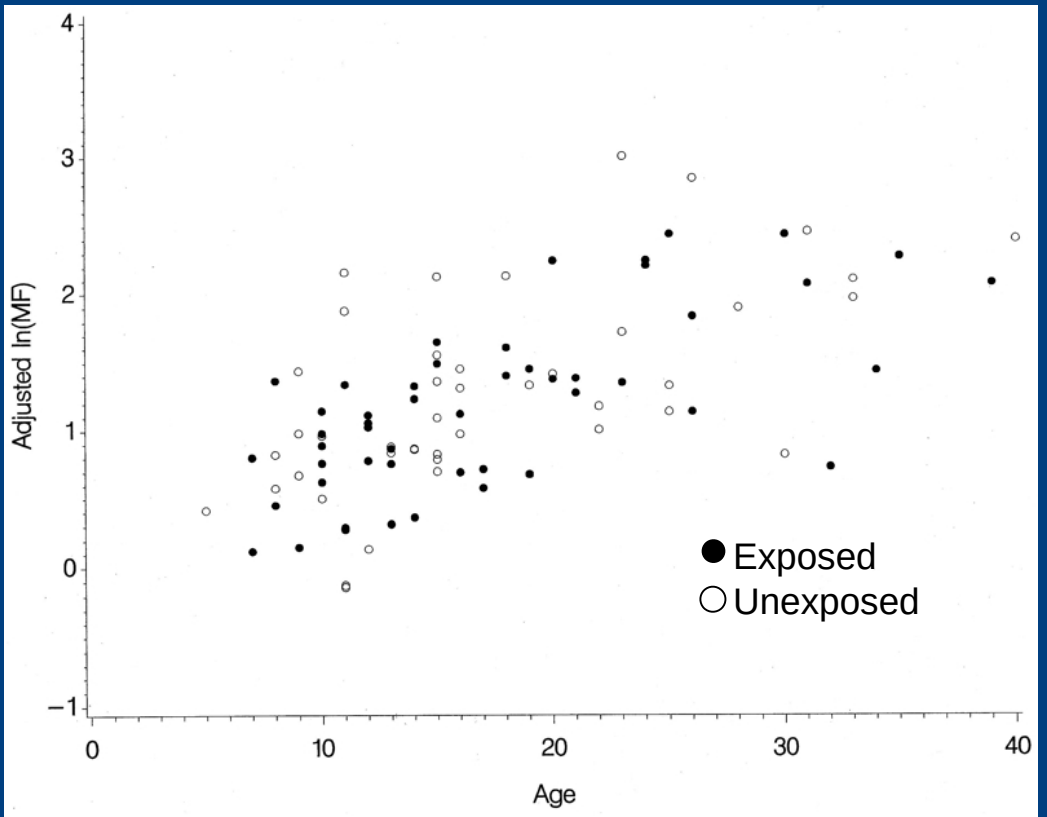
Chemical contaminants came from two industrial sources that have been designated Superfund Sites by the U.S. Environmental Protection Agency, and included aniline- and benzidine-based dyes, epichlorohydrin, trichloropropane substituted anthraquinones and styrene-acrylonitrile trimer, as well as 150 additional chemicals including a particles radionucleotides decay contaminants such as 226Ra, 224Ra, 222Rn and 220Rn

Specific Aim 1

Analysis of the frequency of *HPRT* mutations (Mf) of unexposed children as compared to children exposed to contaminated groundwater from two superfund sites in Toms River N.J.

	Exposed		Controls		p-value
	n	%	n	%	
Gender					
Male	30	61.2%	23	53.5%	0.454
Female	19	38.8%	20	46.5%	
	Mean	S.D.	Mean	S.D.	
Age (yrs)	17.5	7.9	17.4	8.2	0.956
CE	0.55	0.17	0.45	0.15	0.005
Mf x 10-6	3.9	2.85	5.06	4.54	
lnMf x 10-6	1.12	0.7	1.35	0.73	0.135
Residual lnMf	-0.15	0.47	-0.07	0.6	428

Comparative analysis of *HPRT* Mf in Exposed and Control Subjects



The relationship between mutant frequency and age in expose siblings of children with cancer who are from a population with an elevated incidence of childhood cancer (●) and unexposed children from neighboring communities with no increase in cancer incidence (○). lnMf values have been adjusted to the average unselected CE of 50% for all study subjects: adjusted lnMf = lnMf + 1.676(CE – 0.50).

Linear Regression Analysis

	Exposed		Controls		Both Groups		Vermont Data	
	B	S.E.	B	S.E.	B	S.E.	B	S.E.
Intercept	0.992	0.542	1.003	0.645	0.956	0.411	0.074	0.103
CE	1.511	0.0389	0.900	0.634	1.386	0.389	1.484	0.209
ln(Age+1)	1.040	0.166	0.977	-0.214	1.108	0.132	0.765	0.023

Specific Aim 2

To investigate the *HPRT* mutation spectrum of exposed and unexposed groups to determine if genetic differences exist as a biomarker of effect following genotoxic exposure

- We have a repository of 661 *HPRT* mutant clones from 49 exposed children and 647 *HPRT* mutant clones from 43 unexposed children.
- Our double blind approach is to initially perform mutational analysis (RT/PCR, genomic PCR and DNA sequencing) on a total of 460 mutant clones (5 mutant isolates from each subject). This is being done in groups of 10 subjects (5 exposed and 5 controls).
- To date, we have completed the mutational analysis over 150 mutants from 30 subjects.

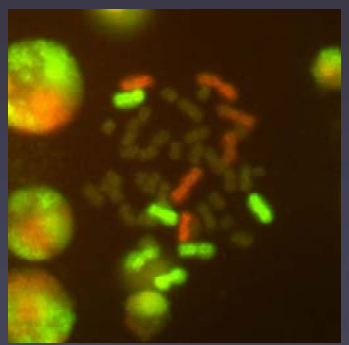
Specific Aim 3

To perform a comparative analysis between the frequency of chromosomal aberrations in peripheral MNC's from exposed children and unexposed controls to investigate the prevalence of widespread genomic damage and its association between genotoxic exposure and cancer risk

- We have a successful approach for making high quality metaphase spreads with 500 - 700 metaphases per slide.

- We are completing our testing of a 2 dye, 6 chromosome for determining the frequency of chromosomal aberrations

- To date, we have made metaphase spreads from 60 of the 92 subjects that will be included in this double blind analysis.



Specific Aim 4

To determine genotype frequencies for 18 metabolic polymorphisms of 11 genes in the exposed children with cancer, their siblings and unexposed controls to determine correlations of genotype, *HPRT* Mf and mutational spectra, and CA's

- The first part of this study is the recruitment of peripheral blood samples for MNC analysis from the affected siblings of those exposed subjects previously enrolled for specific aims 1-3. We have recruited 15 of the 40 blood samples for this study.

Other

We have also developed an new questionnaire for obtaining information about all residences and schools attended by the study participants, to enable us to determine potential exposure to contaminated drinking water, as well questions regarding other potential exposures. This information will be used in conjunction with the reconstruction models developed by ATSDR for water-distribution system serving Dover Township.

Summary

Children residing in Dover Township, NJ between 1979-1995 are an ideal population for evaluating biomarkers of effect because their elevated risk of childhood cancer has been well documented and linked to their exposure to contaminated ground water from two EPA designated superfund sites.

Information on the relationships between exposure, genetic effects and susceptibility obtained from a study of biomarkers would provide valuable biologic insight for interpretation of the epidemiologic findings and for studying the utility of biomarkers for assessing pediatric cancer risk.