The Northern California Childhood Leukemia Study (NCCLS):
10 Years of Experience in Environmental and Genetic Epidemiology

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NIEHS Superfund Basic Research Program
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Childhood Cancers (ages 0-14)

Percent distribution by type

- Leukemias: 35%
- Central Nervous System: 11%
- Other Nervous System: 4%
- Non-Hodgkin's Lymphoma: 6%
- Bone: 4%
- Kidney: 4%
- Soft-tissue sarcomas: 4%
- Hodgkin's disease: 4%
- Retinoblastoma: 4%
- Other: 7%

California Cancer Registry
Childhood Leukemia

Heterogeneous disease with 4 major histologic subtypes

- ALL: 79%
- AML: 12%
- CML: 7%
- Other: 2%

Source: Cancer in California, 1988-1991
Molecular Subsets of ALL

INFANTS
- TEL-AML1
- BCR-ABL
- MLL
- other

CHILDREN
- TEL-AML1
- BCR-ABL
- MLL/11q23
- E2A-PBX1
- Hyperdiploid
- other
Facts About Childhood Leukemia

• Approximately 2,500 new cases per year among children under age 15 years in the U.S.

• Highest incidence rates in
  • Whites
  • Hispanics
  • Males

• Peak incidence of leukemia at age 2-5 years
Total childhood cancer age-specific incidence rates by leukemia vs. non-leukemia (all races, both sexes, 1986-94)
Causes of Childhood Leukemia

• The causes of 90% of childhood leukemias are unknown

• Established risk factors account for only 10%
  - genetic conditions (e.g., Down syndrome)
  - ionizing radiation (*in utero* & postnatal)
  - chemotherapeutic agents
Suspected Risk Factors for Childhood Leukemia

- Residential chemical exposures of parents and/or child
- Chemicals to which parents have been exposed at work
- Tobacco smoke
- Viral infections
- Dietary exposures, especially micronutrients, of parents and/or child
- Non-ionizing radiation exposure of parents and/or child
Schematic Framework for Considering Cancer Etiology in Children

Gene pool

Environmental Exposures

Preconception

Maternal
- Gametes?
- Stored toxins to which fetus exposed in utero or during breastfeeding
  - Example: Trisomy and leukemia

Paternal
- Gametes?

Maternal
- Store toxins to which fetus exposed in utero or during breastfeeding
  - Example: X-rays and leukemia

Pregnancy

Postnatal

- Transplacental
- Not placentally mediated
  - Example: X-rays and leukemia

- Direct exposure of the child
  - Example: Hepatitis B and hepatocellular carcinoma

MOLECULAR DAMAGE

Cancer

GERM CELL MUTATION

Gametes
Conferring genetic predisposition of index child
- Example: Retinoblastoma
Two-hit model

- Gene rearrangements
  - Hallmark of CL
  - But they are not always sufficient for CL → may be the first “hit”

- One or more additional “hits” may be needed
  - Child’s genetic susceptibility
  - In utero exposures (incl maternal effects)
  - Post-natal exposures

From: Greaves M, BMJ, 2002
The NCCLS Objectives

• Examine the relationship between environmental exposures and childhood leukemia
  • Pesticides & chemicals in households & drift from residential areas and parental workplaces, tobacco smoke infectious agents, and diet
  • During critical periods of the child’s development
    • For overall and major molecular leukemia types
    • For White non-Hispanics and Hispanics

• Explore modification of risks by metabolic polymorphisms
Genetics and Environmental Risk Factors in Childhood Leukemia

- Environmental Factors
- Genetic Factors
- Most likely a Gene-Environment Interaction

Exposure

Genetic Risk

Benzene
Infection
Diet

Leukemia
The NCCLS Design

• Population-based case-control study
• Started in 1995 – End of enrollment in 2008
• Network of 9 pediatric oncology centers in 35 counties in Northern and Central California
• Inclusion of Hispanic population (47%)
• Multi-disciplinary team
  - Pediatric oncologists, epidemiologists, molecular biologists, nutritionists, toxicologists, and industrial hygienists
• Primarily funded by the National Institute Environmental Health Sciences
NCCLS Study Area

- 17 Bay Area counties
- 18 Central Valley counties
NCCLS Case Eligibility Criteria

• New diagnosis of leukemia
• Biological parent speaks English or Spanish
• No previous cancer diagnosis
• Resident of study area at time of diagnosis
Case Enrollment

• Incident cases rapidly ascertained within 48 hrs
  - Obtain informed consent
  - Collect pre-treatment blood and/or bone marrow specimens within 72 hours in 86%

• NCCLS identified over 88% of incident leukemia cases, compared with California Cancer Registry data (1997-1999).
Case Participation

• As of November, 2005, 1243 cases have been ascertained.
• Of these, 960 (77%) are eligible to participate.
• 829 (86%) eligible patients have consented to participate, and 678 have completed interviews.
Cytogenetic Classification

Childhood Leukemia
N=509

11q23/MLL
4% (n=20)

ALL
(without MLL)
81% (n=410)

AML
(without MLL)
14% (n=73)

CML
(without MLL)
0.8% (n=4)

Other
(without MLL)
0.4% (n=2)

ALL
(n=10)

AML
(n=10)

B cell
lineage
88% (n=360)

T cell
lineage
9% (n=36)

Biphenotypic
1% (n=3)

Unknown*
3% (n=10)

*missing immunophenotype information
Cytogenetic Classification

B cell ALL without \textit{MLL} \\
N=360

- High hyperdiploid 51-67 \\
  \hspace{0.5cm} 37\% \\
  \hspace{0.5cm} (n=134)
  - t(12;21) \\
    \hspace{0.5cm} 20\% \\
    \hspace{0.5cm} (n=71)
  - Pseudodiploid \\
    \hspace{0.5cm} 3\% \\
    \hspace{0.5cm} (n=12)

- Low hyperdiploid 47-50 \\
  \hspace{0.5cm} 10\% \\
  \hspace{0.5cm} (n=36)

- t(1;19) \\
  \hspace{0.5cm} 4\% \\
  \hspace{0.5cm} (n=13)

- Diploid \\
  \hspace{0.5cm} 18\% \\
  \hspace{0.5cm} (n=63)

- Hypodiploid 30-45 \\
  \hspace{0.5cm} 4\% \\
  \hspace{0.5cm} (n=16)

Prognosis

- Favorable (60\%) 
- Intermediate (28\%) 
- Poor (8\%)

Not shown: cases with unknown prognosis (n=15)
NCCLS Control Selection

- Concurrent to case ascertainment
- Achieved using California Birth Registry and electronic tracing technologies
- Individually matched to case by:
  - Date of birth, gender, maternal race, Hispanic status, mother’s county of residence at child’s birth
  - 1 or 2 controls per case
Control Participation

• The number of searches conducted for each participating control ranges from 1 to 16, with an average of 2.7.
• Approximately 66% of participating controls are first choice or “ideal” controls.
• Assess the representativeness of participating controls to the source population, by comparing socio-demographic characteristics between the participating and non-participating controls.
Collection of Interview Data

• In-person computerized assisted interview
  - Biological parents (mostly mothers)
  - English or Spanish

• Comprehensive questionnaire
  - Detailed time-specific exposure assessment
  - Mother and child’s diet
  - Daycare attendance and childhood infections
  - Residential history (=> geocoding)
  - Parental smoking
  - Parental occupation
    • Job titles
    • 19 task-specific questionnaires adapted from NCI job modules (no surrogate interviews)
  - Household chemical use
Collection of Biospecimens

• Blood and bone marrow specimen: 86% cases
  - Using proteomics to classify leukemia into molecular subgroups
  - RAS mutation

• Buccal cells in case & control children and their mothers: 98%
  - Genetic polymorphisms

• Archived Newborn Blood specimens (Guthrie cards) for case & control children: 85%
  - Backtracking to birth of chromosome translocations in cases: t(12;21), t(8;21), t(15;17), inv (16)

• New: maternal blood/urine specimens
  - Collaboration with CDC-National Center for Environmental Health for analyses of chemicals and folate levels
  - Use of protein adducts as a biomarker of exposure
Genotyping in the NCCLS

- DNA extracted from buccal cells and ANB specimens and amplified to permit assaying of 1000’s of SNPs
  - Examine effects of child’s own genetic susceptibility
  - Examine in utero effects related to maternal genes

- Focus on candidate genes encoding enzymes involved in important pathways (e.g., protecting from environmental insults, cell growth and regulation):
  - Xenobiotic metabolism and transport enzymes (exogenous substances, including chemicals, pesticides, benzene, pollutants)
  - Metabolism of nutrients including folate, other vitamins, growth factors
  - Antioxidant enzymes
  - DNA repair enzymes
  - Immune function
Genotyping in the NCCLS

- Preliminary genotyping on ~20 SNPs in a limited group of samples
- Plan to type all samples (Illumina)
  - ~1000 cases, ~1500 controls
  - ~200 genes
  - Birth mothers (~2500)
  - Case fathers
- Goals
  - Child susceptibility
  - Maternal-child effects
  - Genetic transmission to case children from mothers and fathers
Collection of Environmental Samples

• Follow-up-visit within 3 to 9 months
  - Children age \( \leq 7 \) yrs; same residence as diagnosis or reference date
  - Reliability study on household chemical use
  - Air, dust, and window wipe sampling
Multi-Step Approach to Characterize Exposure to Pesticide

**ELIGIBILITY**

- CHILD AGE < 8 YEARS AND LIVING AT CURRENT RESIDENCE SINCE REFERENCE DATE
- ALL ELIGIBLE FOR THE STUDY

**RESEARCH QUESTIONS**

- "What levels of the relevant compounds are present in the home environment currently?"
- DUST & WINDOW WIPE SAMPLING
- MATERNAL URINE

- "Is the active ingredient (compound) present in the home?"
- 2ND INTERVIEW and DIRECT INVENTORY

- "What products do people report using from preconception to age 3 years?"
- 1ST INTERVIEW

**STEP 1**

IN-PERSON INTERVIEW

**STEP 2**

HOUSEHOLD SURVEY - RELIABILITY

**STEP 3**

MEASUREMENT
Environmental Home Sampling

• Commenced in 2002
• About 50% of case and control families
• Collection of **dust samples**
  • Collaboration with NCI
  • 380 samples as of October 31, 2005
  • Current analysis of pesticides, polychlorinated biphenyls, & nicotine
• Collection of **air samples**
  • 355 samples as of October 31, 2005
  • Current analyses of benzene & toluene
• Target = 489 case and control homes
Dust sample collection during home visit

High Volume Surface Sampler (HVS3) vacuum
Pesticides
Multiple Sources of Exposure

- **Environmental**
  - Parental workplace
  - Drift from nearby agricultural areas
  - Home use
  - School use
- **Dietary**
  - Water
  - Food
  - Breastfeeding
1991-1994 Annual Average Pesticide Use

1999 Respondents

- **8,189 - 847,991 lbs.**
- **2,884 - 8,189 lbs.**
- **537 - 2,884 lbs.**
- **33 - 537 lbs.**
- **0 - 33 lbs.**

Bay Area Counties
Central Valley Counties

50 0 50 100 150 Miles
Previous Studies of Childhood Leukemia and Pesticide Exposure

• Agricultural use
  - Ecologic & case-control studies in California (Reynolds, 2002 & 2005)
    • No association with childhood cancers
    • Suggestion of 50% increased risk of leukemia in children exposed to propargite, an insecticide used in orchard & vineyards

• Parental occupations
  - Several studies showing increased leukemia risk, but questionable exposure assessment
  - Increased incidence of cancers and lymphomas in children of the pesticide applicators enrolled in the NCI Agricultural Health Cohort Study (Flower K, 2004)

• Home use
  - Canadian (Infante-Rivard C, 1999) and French (Menegaux F, 2005) studies with similar design as the NCCLS reported increased risks with use of home and garden insecticide during pregnancy and childhood
Model for Pesticide Exposure Assessment in the NCCLS

- **Self-reports**
  - Residential history
  - Pesticide use at home
  - Parental occupational history

- **Home dust samples**

- **GIS attributes**
  - Linkage to the Pesticide Use Registry, CA
Model for Pesticide Exposure Assessment

- **Self-reports**
  - Residential history
  - Pesticide use at home
  - Parental occupational history

- **Home dust samples**

- **GIS attributes**
  - Linkage to the Pesticide Use Registry, CA
Figure 1. Indoor and outdoor pesticide exposures and the risk of childhood ALL. The box ORs; vertical bars reflect upper and lower limits of 95% CIs.

*Adjusted for annual household income.

Source: Ma X, et al. Environmental Health Perspectives. v.110, no.9, September 2002
# Pesticide Use From Preconception up to 3 Years Post-natally in 382 Children With Leukemia and 482 Controls

<table>
<thead>
<tr>
<th>Type of exposure</th>
<th>Cases/controls</th>
<th>OR (^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Combined exposures</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indoor insecticides</td>
<td>299/353</td>
<td>1.5 (1.1-2.1)</td>
</tr>
<tr>
<td>Outdoor pesticides</td>
<td>136/171</td>
<td>1.2 (0.9-1.7)</td>
</tr>
<tr>
<td>Outdoor herbicides</td>
<td>159/189</td>
<td>1.5 (1.1-1.9)</td>
</tr>
<tr>
<td><strong>Selected individual exposures</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Professional pest control</td>
<td>110/122</td>
<td>1.4 (1.0-2.2)</td>
</tr>
<tr>
<td>Professional lawn services</td>
<td>74/73</td>
<td>1.6 (1.1-2.3)</td>
</tr>
<tr>
<td>Insecticides</td>
<td>241/281</td>
<td>1.4 (1.0-1.8)</td>
</tr>
<tr>
<td>Slug/snail baits</td>
<td>74/102</td>
<td>1.1 (0.7-1.6)</td>
</tr>
<tr>
<td>Rodenticides</td>
<td>59/67</td>
<td>1.3 (0.8-1.9)</td>
</tr>
<tr>
<td>Products for weeds</td>
<td>109/138</td>
<td>1.3 (0.9-1.9)</td>
</tr>
<tr>
<td>Indoor foggers for fleas</td>
<td>65/55</td>
<td>1.5 (1.0-2.2)</td>
</tr>
</tbody>
</table>

\(^1\) The odds ratios are derived from conditional logistic regression, adjusted for household income; numbers in parentheses are 95% confidence intervals.
<table>
<thead>
<tr>
<th>Number of products</th>
<th>Cases/controls</th>
<th>OR&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>51/86</td>
<td>1.0</td>
</tr>
<tr>
<td>1</td>
<td>74/95</td>
<td>1.5 (0.9-2.4)</td>
</tr>
<tr>
<td>2</td>
<td>72/98</td>
<td>1.5 (0.9-2.4)</td>
</tr>
<tr>
<td>3</td>
<td>64/82</td>
<td>2.0 (1.2-3.3)</td>
</tr>
<tr>
<td>4</td>
<td>62/53</td>
<td>3.1 (1.7-5.6)</td>
</tr>
<tr>
<td>5 or more</td>
<td>59/68</td>
<td>2.4 (1.4-4.2)</td>
</tr>
</tbody>
</table>

<sup>1</sup> OR=odds ratios adjusted for annual household income; Numbers in parentheses are 95% confidence intervals.

Similar dose-response relationships were observed separately for pesticide used during pregnancy and after birth, but not before conception.
## Pre- & Post-Natal Use of Indoor Insecticides

**Exclusive or Combined Time Window of Exposure**

<table>
<thead>
<tr>
<th>Time window</th>
<th>Cases/controls</th>
<th>OR&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never</td>
<td>53/97</td>
<td>1.0</td>
</tr>
<tr>
<td>Only before birth</td>
<td>29/17</td>
<td>2.9 (1.5-5.7)</td>
</tr>
<tr>
<td>Only after birth</td>
<td>86/115</td>
<td>1.3 (0.9-2.0)</td>
</tr>
<tr>
<td>Both before &amp; after birth</td>
<td>185/216</td>
<td>1.7 (1.1-2.4)</td>
</tr>
</tbody>
</table>

<sup>1</sup> OR=odds ratios adjusted for annual household income; Numbers in parentheses are 95% confidence intervals.
Focus on Pre-Natal Use of Indoor Insecticides: Exclusive or Combined Time Window of Exposure

<table>
<thead>
<tr>
<th>Time window</th>
<th>Cases/controls</th>
<th>OR¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never</td>
<td>159/231</td>
<td>1.0</td>
</tr>
<tr>
<td>Only before pregnancy</td>
<td>16/30</td>
<td>0.7 (0.4-1.4)</td>
</tr>
<tr>
<td>Only during pregnancy</td>
<td>78/65</td>
<td>1.9 (1.3-3.0)</td>
</tr>
<tr>
<td>Both before &amp; during pregnancy</td>
<td>129/156</td>
<td>1.5 (1.1-2.1)</td>
</tr>
</tbody>
</table>

¹ OR=odds ratios adjusted for annual household income; Numbers in parentheses are 95% confidence intervals.
Conclusions

• Strong evidence that *in utero* and post-natal exposures to *indoor insecticides* are critical in the development of childhood leukemia.
  - No association is observed with preconception use

• Similar analyses conducted for *outdoor herbicides* showed increased risks with pre- and postnatal exposures
  - Numbers are limited to evaluate separate roles of preconception and *in utero* exposures.

• Associations mainly observed for *ALL* and *Hispanic* children, although differences by histologic type (*ALL* and *AML*) and ethnic group are not statistically significant with the current sample size.
Future Directions

• Refine assessment of environmental exposures.
  – Integrate other sources of pesticide exposure, such as drift or “take home” chemicals from outdoor areas and workplaces of parents.
  – Measure levels of selected pesticides in house dust and maternal urine samples.
  – Complete analyses on reliability of self-reports

• Identify genetic polymorphisms involved in the metabolism of pesticides.
  - E.g., PON1 gene polymorphism and organophosphate metabolism.

• Increase sample size.
  - Analyze by type of pesticide, by histologic and molecular subtype of leukemia, and by ethnic group.
NCCLS Strengths

- Large sample size – Expected total = 1000 cases
- Comprehensive and detailed chemical exposure assessment
- Strong genetic and molecular components
- Research team able to evaluate environmental and genetic factors simultaneously
NCCLS Investigators

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Katherine Hammond, Ph.D., School of Public Health, UC Berkeley
Marilyn Kwan, Ph.D., Division of Research, Kaiser Permanente
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Xiaomei Ma, Ph.D., Yale University School of Medicine
Catherine Metayer, Ph.D., School of Public Health, UC Berkeley
Peggy Reynolds, Ph.D., California Department of Health Services
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Luoping Zhang, Ph.D., School of Public Health, UC Berkeley

Danit Aharon, Melinda Aldrich, Jeffrey Chang, Neela Guha, Jill Hardin, Kevin Urayama, Graduate Student Researchers, School of Public Health, UC Berkeley
NCCLS Collaborating Hospitals and Grants

- Children’s Hospital Oakland
- Kaiser Permanente Medical Group in Oakland, San Francisco, Sacramento, Santa Clara
- UCSF School of Medicine
- Children’s Hospital of Central California, Fresno
- Stanford University Lucille Packard Children’s Hospital
- UC Davis School of Medicine

Childhood Leukemia (Grant: 2RO1ES09137-06)
Superfund (Grant: P42 ES04705-18)
National Cancer Institute (Westat Contract # 015619)
Thank to the families participating in the NCCLS
## NCCLS pesticide exposure methods

- Partial list of the 50 pesticides measured in carpet dust:

<table>
<thead>
<tr>
<th>Lawn &amp; garden Crop herbicides</th>
<th>Insecticides</th>
<th>Fungicides</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4-D</td>
<td>Chlordane</td>
<td>Ortho-phenylphenol</td>
</tr>
<tr>
<td>MCPA</td>
<td>DDE + DDT</td>
<td></td>
</tr>
<tr>
<td>Dicamba</td>
<td>Dieldrin</td>
<td></td>
</tr>
<tr>
<td>Trifluralin</td>
<td>Methoxychlor</td>
<td></td>
</tr>
<tr>
<td>Simazine</td>
<td>Heptachlor</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Carbaryl</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chlorpyrifos</td>
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<tr>
<td></td>
<td>Diazinon</td>
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<td></td>
<td>Malathion</td>
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<td></td>
<td>Propoxur</td>
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