Session 5

Environmental Risk Factors: Pesticides

ICNIRP/WHO/BfS International Workshop
Risk Factors for Childhood Leukemia

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Multiple Sources of Child's Exposure to Pesticides

- Environmental
  - ✓ Home use +++
  - ✓ Drift from agricultural areas
  - ✓ "Take home" from parental workplace
  - Exposure at school
- Dietary (mother during pregnancy and/or child)
  - Water
  - Food
  - Breastfeeding

Diverse Chemical Structures

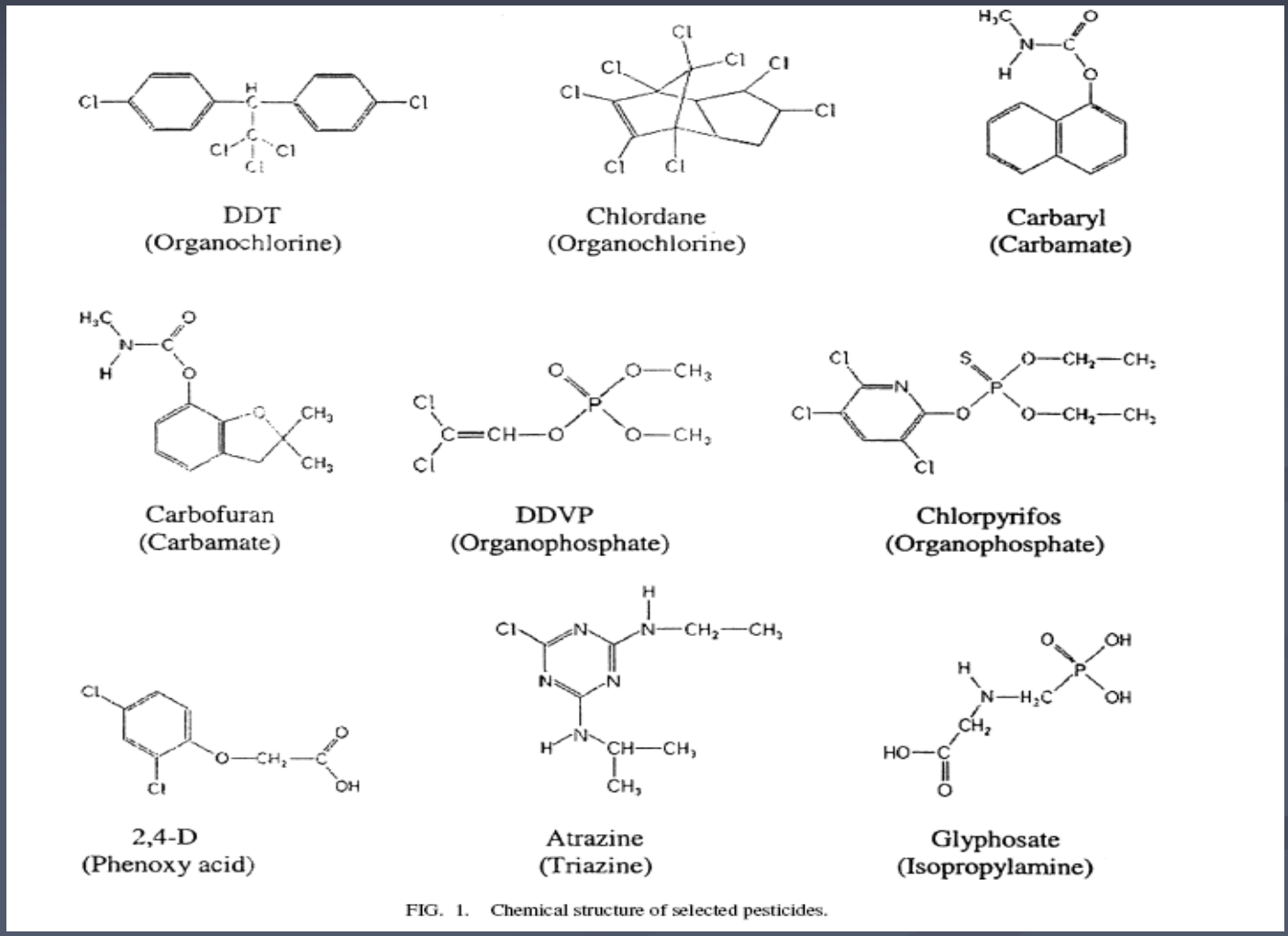


FIG. 1. Chemical structure of selected pesticides.

OVER 800 REGISTERED ACTIVE INGREDIENTS IN THE US

Challenges to Establish Human Carcinogenicity of Pesticides

- Large number to be tested
- Complex mixture
- Short-term bioassays in rodents commonly used, but animal studies may not be adequate to evaluate human carcinogenicity
- Possible non-genotoxic mechanisms (not altering directly DNA) may lead to cancer
  - immunotoxicity, epigenetic effect, mutation of parental germ cells

Carcinogenicity?

- IARC (1991) has classified
  - Arsenical insecticides and herbicide TCDD are classified as "human carcinogens, category 1".
  - "occupational exposure in spraying and application of non-arsenical insecticides" as a group as "probable human carcinogens, category 2A".
  - Chlordane, DDT "possible human carcinogens, category 2B".

Carcinogenicity?

- US Environmental Protection Agency (EPA) Office of Pesticide Program provides a more extensive list of pesticides evaluated for carcinogenic potential (1986, 1996, and 1999 classifications)

However, most pesticides on the market today are lacking sufficient scientific evaluation.

DNA Damages in Adults

- DNA damages in human lymphocytes
  - in *vitro* studies
  - among workers using agricultural pesticides
- Biomarkers of early effects
  - chromosomal aberrations & oxidative damages after domestic use

Active Ingredients Versus Other Ingredients

- Commercial pesticides include a mixture of active ingredients (information publicly available) and other ingredients (restricted access)
  - Most laboratory studies have tested active ingredients
  - Limited understanding of the effects of so-called “inert” ingredients
- Some evidence that commercial formulations cause more biological effects than pure active ingredients
  - e.g., herbicide glyphosate (Richard et al. 2005), pyrethroid bifenthrin (Hoffman et al. 2006)

Vulnerability of Children

- High risk behavior indoor and outdoor
  - Hand-to-mouth contact (floor, soil, toys)
  - Breathing zone
  - Dermal absorption?
- Intake and body burden
  - Different metabolism than adults
  - Renal & hepatic capacities vary from infancy to early childhood
  - Higher respiration rates
  - Greater ratio of surface area to body mass
  - Lesser ability to store toxicant in fat may lead to higher circulating levels of toxic agents

Child's Exposure to Pesticides Under Investigation in Epidemiologic Studies

- ✓ Residential use in case-control studies => use of self-reports
- Agricultural pesticides assessed with GIS technology in ecological or case-control studies
- Parental occupational exposure to pesticides in case-control studies

12 Case-control Studies

1987-2007

Study, Year	Location	Disease	Age	Cases
1. Rudant et al., 2007	France	ALL; AML	0-14	646;100
2. Menegaux et al., 2006	France	Acute leuk.	0-14	280
3. Alderton et al., 2006	US	ALL; AML	0-19 (DS)	97;61
4. Ma et al., 2002	US	Leuk.; ALL	0-14	162;135
5. Alexander et al., 2001	International	ALL/AML *(MLL gene)*	Infant	136
6. Meinert et al., 2000	Germany	Leukemia	0-14	1,184
7. Infante-Rivard et al., 1999	Canada	ALL	0-9	491
8. Meinert et al., 1996	Germany	Leukemia	1-14	173
9. Schwartzbaum al., 2002	US	ALL; ANLL	0-14	522;107
10. Leiss et al., 1995	US	Leukemia	0-14	N/A
11. Buckley et al., 1989	US	ANLL	1-18	204
12. Lowengart et al., 1987	US	Leukemia	0-10	123

Study Designs

- Case ascertainment
  - hospitals, cancer registries, clinical trials
- Control selection
  - hospitals, population-based registries, RDD
- Matching
  - individual, frequency
- Interview
  - phone, in-person
- Various pesticide grouping

Associations by Type of Pesticides

(Ever vs. Never and/or dose-response relationship*)

Study, Year	Any	Home	Garden	Herbicides	Fungicides
1. Rudant et al., 2007	X	X	X	X	-
2. Menegaux et al., 2006	X	X	X	(x)	X
3. Alderton et al., 2006 (DS)	X	X*	-	-	
4. Ma et al., 2002	X	X*	-	(x)	
5. Alexander et al., 2001 (IL)	X	X			
6. Meinert et al., 2000	-	-	-		
7. Infante-Rivard et al., 1999	X	X*	X	X	
8. Meinert et al., 1996	X	-	X		
9. Schwartzbaum al., 2002	X				
10. Leiss et al., 1995	X	X	-		
11. Buckley et al., 1989	X				
12. Lowengart et al., 1987	X	X	X		

Associations by Period of Exposure

Study, Year	Not Reported	Pre-Conception	Pregnancy	After Birth
1. Rudant et al., 2007			X	X
2. Menegaux et al., 2006			X	X
3. Alderton et al., 2006			X	-
4. Ma et al., 2002		X	X	X
5. Alexander et al., 2001			X	
6. Meinert et al., 2000				-
7. Infante-Rivard et al., 1999			X	X
8. Meinert et al., 1996	X			
9. Schwartzbaum al., 2002	X			
10. Leiss et al., 1995			X	X
11. Buckley et al., 1989			X	
12. Lowengart et al., 1987	X			

Magnitude of the Associations

- ORs = 1.5 - 2.5
- Associations reported for individual products
  - Problem of multiple comparisons, correlation
- Separate contribution of home vs. garden pesticides not well characterized
  - Higher risk for herbicide use only reported in the French study (Rudant et al., 2007)
- Separate contribution of pre- vs. post-natal exposure not well characterized
  - Higher risk for pre-natal exposure to pesticides (Ma et al., 2002)

Genetic Susceptibility

- Interaction between *CYP1A*1 gene polymorphism AND childhood exposure to pesticides (Infante-Rivard et al. 1999)
- Interaction between *MDR*1 gene polymorphism AND exposure to indoor pesticides (Urayama et al., 2007)
- More genes to study: *PON1*, *GST*s, DNA repair genes

Risk by Cytogenetic Subtype

- Alexander et al. (2001) shows that risk associated with residential pesticide use was higher for infant leukemia with *MLL* gene fusion (OR=5.0, 1.7-14.4) than those without (OR=1.9, 0.4-9.6)
- Next generation studies will look at major cytogenetic subtypes, i.e., t(12:21), hyperdiploidy, etc…
  - Critical to work with the Childhood Leukemia International Consortium (CLIC) to achieve adequate sample size

Concluding Remarks

- Studies from different countries and with different designs have consistently reported associations of small to moderate magnitude between childhood leukemia (ALL mostly studied) and self-reported use of home and/or garden pesticides used before and/or after birth.
- Specificity?
  - WHAT pesticides are important risk factors?
- Temporality?
  - WHAT time windows of exposure are critical?
- Genetic predisposition?
  - Several studies are currently underway to examine the effects of genes involved in transport and metabolism of pesticides.

Use of Self-reports

- Participants *can* report pest treatments in the home and garden by type of pest treated.

  Colt et al. reported good correlation between carpet dust concentration of pesticides and self-reports in an adult NHL study.

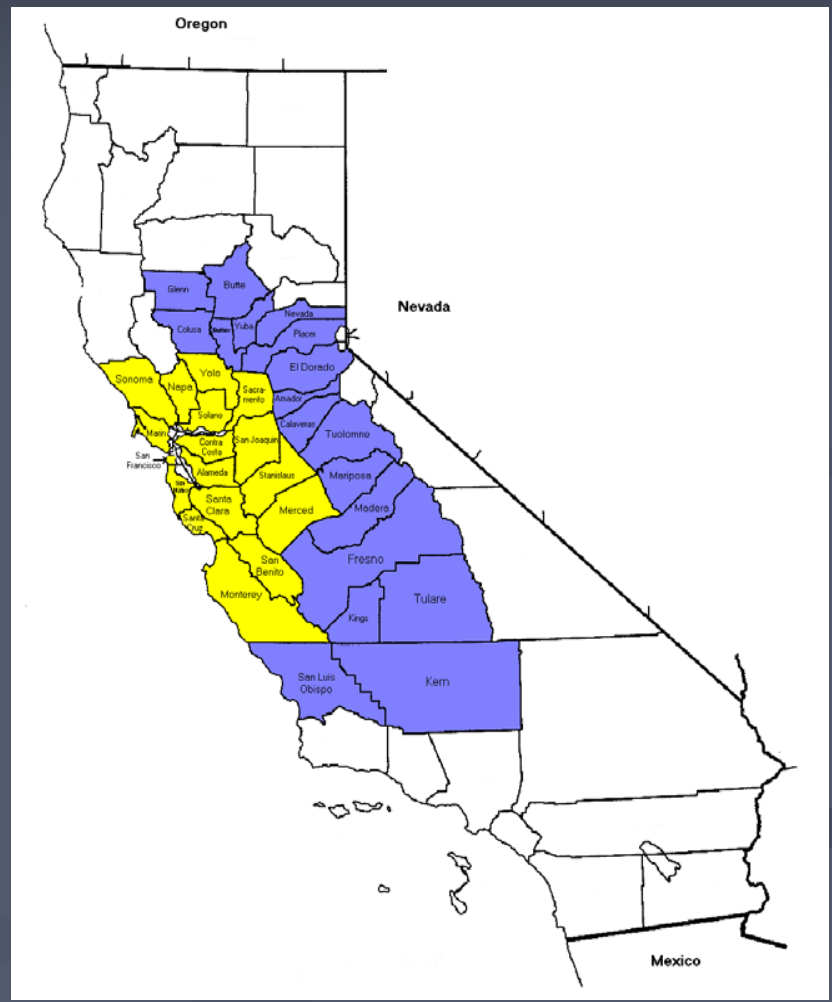
- Self-reports provide time-specific exposure (preconception, pregnancy, after birth)

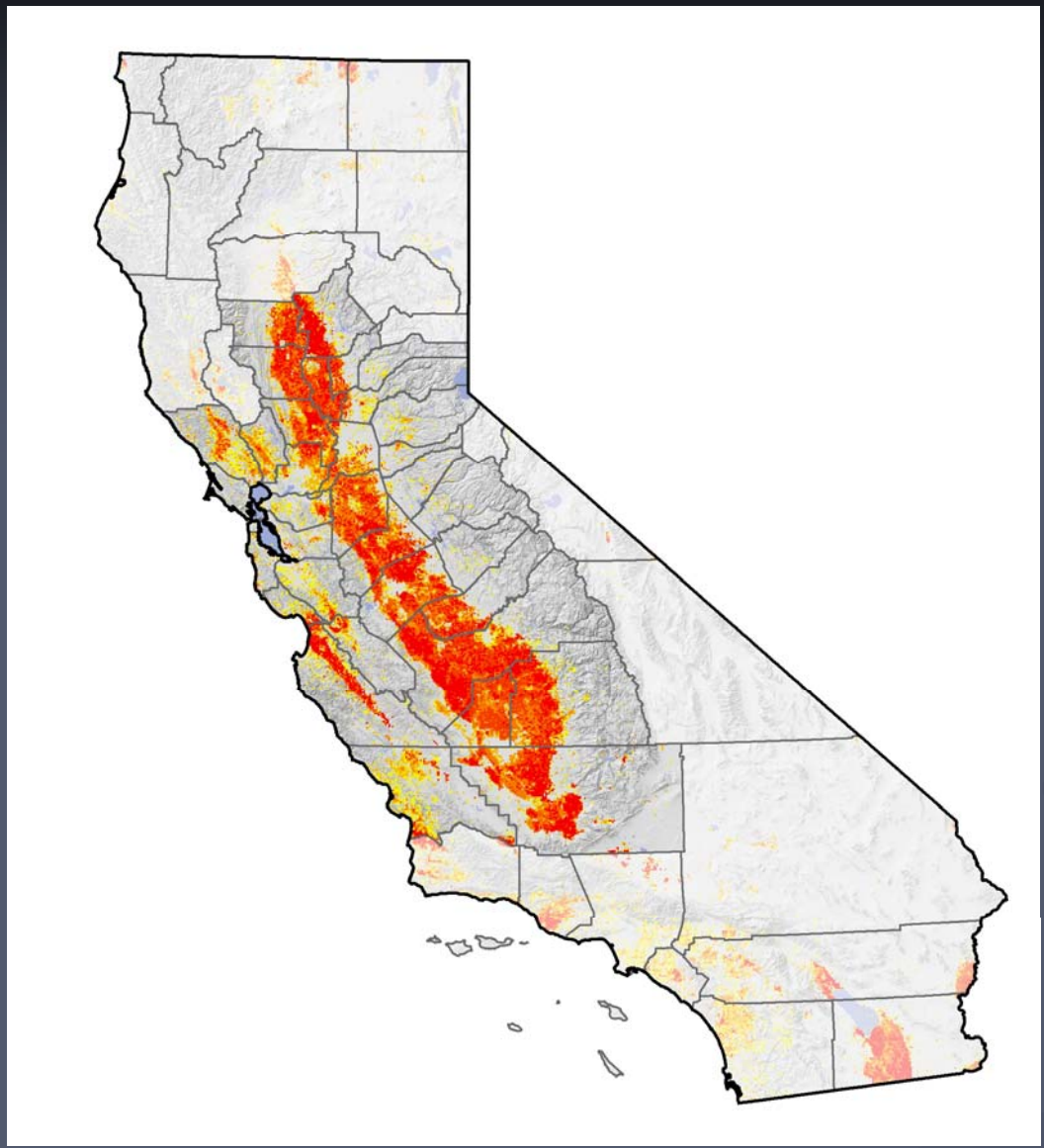
Limitations

- Most people cannot remember product names, do not know specific active ingredients.
- Parents of child with cancer may have better recall.
  (However, magnitude of associations varies for childhood AL, NHL, HD, and histologic subtypes)
- Pesticides used near home cannot be determined from interviews.

Experience from the Northern California Childhood Leukemia Study (NCCLS)

- Population-based case-control study
- 1995–2009
- 1,000 cases – 1,300 controls
- 35 California counties





Pounds of Pesticides Applied (Probable/Possible Carcinogens)

1990-2003

Class B & C Carcinogens

Annual Average 1990-2003

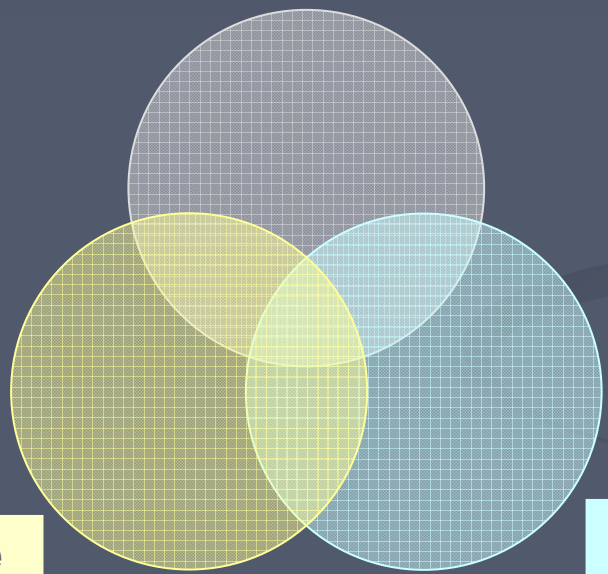
Quintiles (lbs.)

1,491.46 -	117,902.01
594.69 -	1,491.45
122.53 -	594.68
7.81 -	122.52
0.01 -	7.80

Source: Rull R. Northern California Cancer Center

Model for Pesticide Exposure Assessment in the NCCLS

Interviews – all subjects
Pesticides at home
Parental occupations
Residential history



2nd Home visit in stable residences
2nd interview
Product inventory
Dust samples

GIS attributes
Linkage to the CA environmental Database for Agricultural Pesticides

Key Findings

- ✓ Interviews and reproducibility
- ☐ Pesticide inventory
- ☐ Home dust sampling

Indoor Insecticide Use and Risk of ALL (n=441): From Preconception up to 3 Years Old

	Cases/Controls	OR[1]
Insecticides		
Professional pest control	142/165	1.3 (0.95-1.72)
Insecticides (crawling/flying)	297/343	**1.4 (1.07-1.86)**
Insect repellents	110/132	1.2 (0.84-1.59)
Indoor foggers for fleas	78/68	1.4 (0.97-2.01)

[1] The odds ratios are derived from conditional logistic regression, adjusted for household income; numbers in parentheses are 95% confidence intervals.

(Metayer C. Submitted)

Outdoor Pesticide Use: ALL (n=441)

From Preconception up to 3 Years Old

	Cases/Controls	OR[1]
Herbicides		
Professional lawn services	99/96	**1.7 (1.18-2.30)**
Products for weeds	135/171	1.3 (0.95-1.76)
Other pesticides		
Slug/snail baits	90/121	1.1 (0.80-1.60)
Rodenticides	71/81	1.2 (0.83-1.71)
Insect/disease of plants/trees	62/81	1.1 (0.75-1.61)

[1] The odds ratios are derived from conditional logistic regression, adjusted for household income; numbers in parentheses are 95% confidence intervals.

(Metayer C. Submitted)

Dose-response

	Cases/Controls	OR[1]
Insecticides		
None	43/78	1.0 (ref)
1	82/107	1.4 (0.89-2.36)
2	89/113	1.6 (0.98-2.58)
3	85/104	2.0 (1.18-3.25)
4	63/68	2.2 (1.28-3.87)
5+	79/86	2.4 (1.06-1.23)
		p-trend <0.05

[1] The odds ratios are derived from conditional logistic regression, adjusted for household income; numbers in parentheses are 95% confidence intervals.

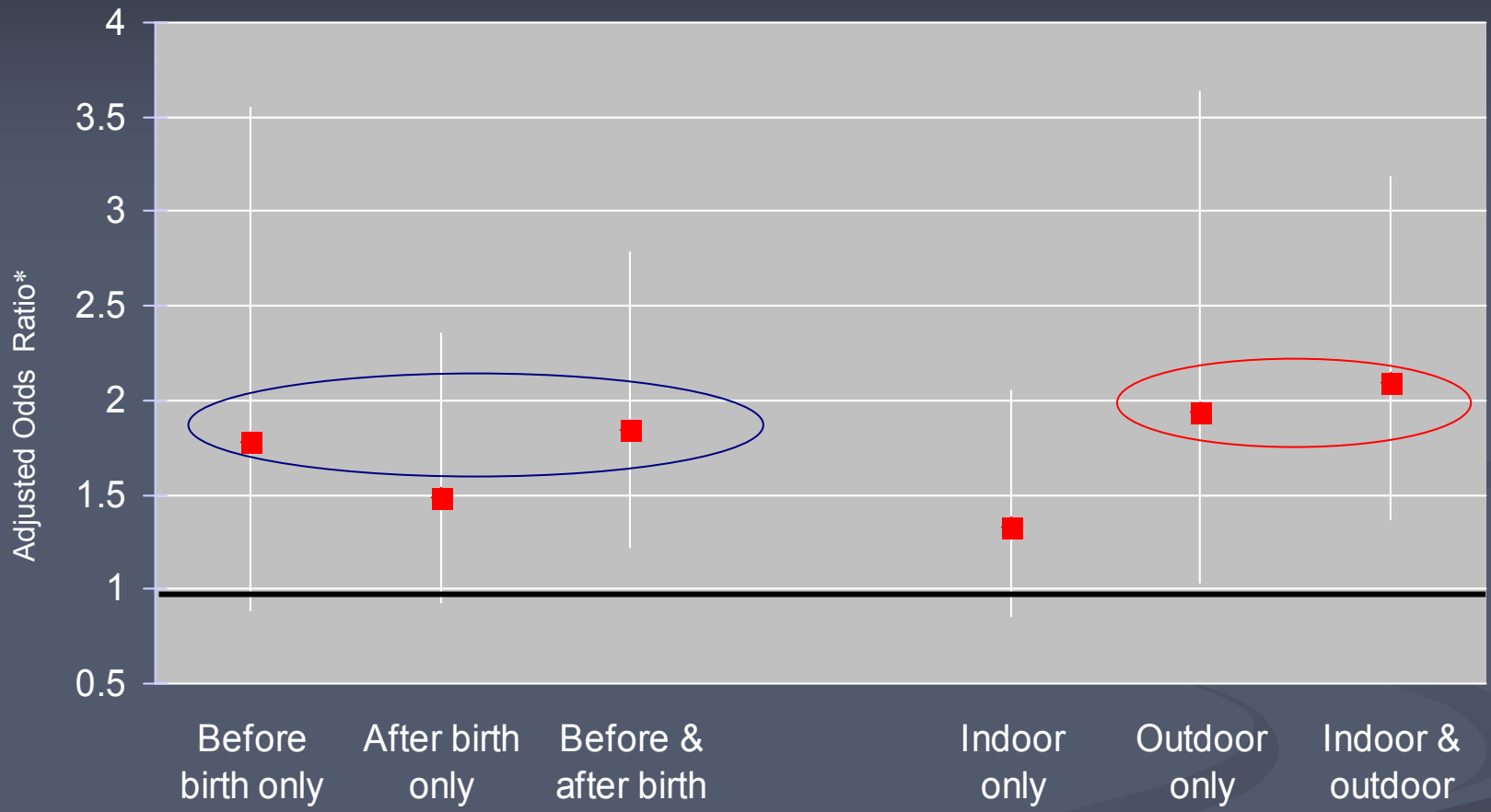
(Metayer C. Submitted)

Characteristics of Pesticide Use

- What?, Where?, When?, How much?
  - ⇒ Correlated data

- We used Principal Components Analysis, a useful data summarization technique that creates independent factors
  - Quantity
  - Type (insecticides, herbicides, other pesticides)
  - ✓Location (indoor, outdoor), $p \leq 0.01$
  - ✓Period (before and after birth), $p = 0.01$

Period and Location of Pesticide Use



*Adjusted for annual household income

Reproducibility of Self-reported Use of Home Pesticides (~450 households)

	Ppos[1] (95% CI)		Pneg[2] (95% CI)	
	Cases	Controls	Cases	Controls
Flea, tick	0.70(0.61-0.78)	0.73(0.65-0.80)	0.83(0.78-0.88)	0.83(0.79-0.88)
Ant, fly	0.80(0.75-0.85)	0.77(0.72-0.82)	0.55(0.45-0.66)	0.54(0.44-0.64)
Indoor fogger	0.47(0.28-0.67)	0.49(0.31-0.67)	0.95(0.92-0.97)	0.94(0.92-0.97)
Weed/plant	0.74(0.66-0.82)	0.80(0.74-0.86)	0.79(0.72-0.85)	0.77(0.71-0.84)
Pest control	0.70(0.61-0.79)	0.71(0.62-0.79)	0.86(0.81-0.90)	0.86(0.82-0.90)
Lawn service	0.71(0.61-0.81)	0.62(0.49-0.75)	0.90(0.87-0.94)	0.93(0.90-0.96)
Mean difference[3] (95% CI)	0.028 (-0.013, 0.043)		0.008 (-0.011, 0.003)	
p-value[4]	0.155		0.863	

[1] Ppos = proportion positive agreement. [2] Pneg = proportion negative agreement.

[3] Bootstrapped difference of means between cases and controls.

[4] Bootstrapped p-value.

Overall good concordance over time

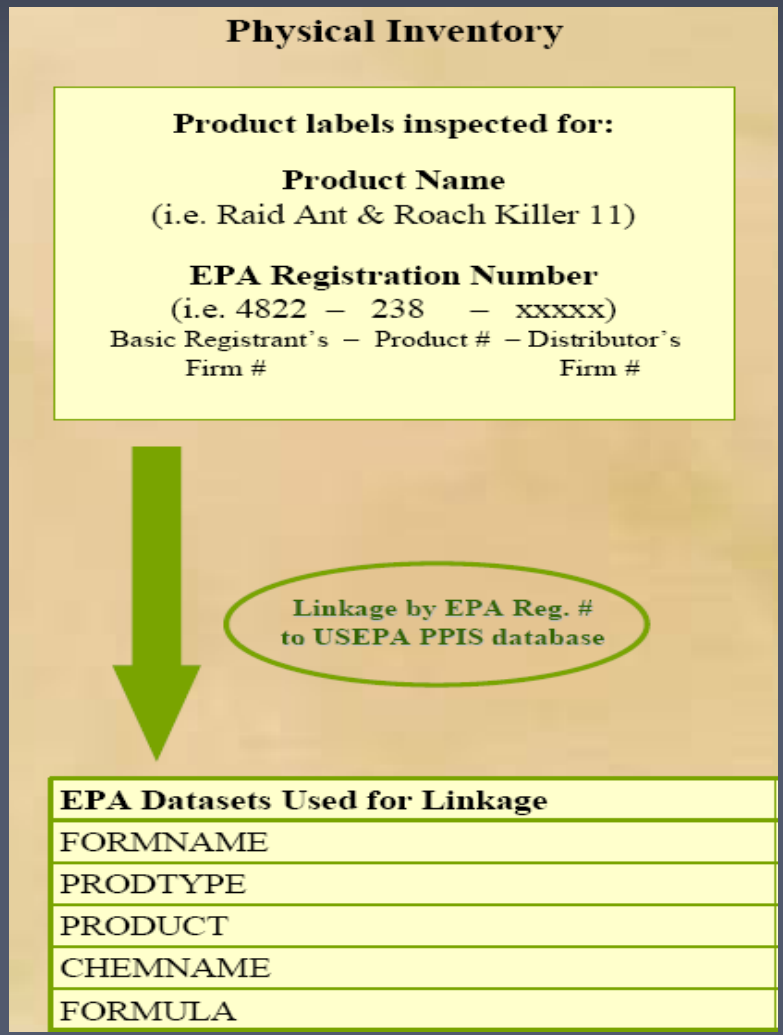
No difference between cases and controls

Source: Aharon et al. ISEE Conference, Paris, 2006 (Abstract)

Key Findings

- Interviews and reproducibility
- ✓ Pesticide inventory
- Home dust sampling

Linkage to US-EPA Database



Objective: to conduct a survey of active ingredients present in the NCCLS homes

=> semi-qualitative evaluation to complement interviews

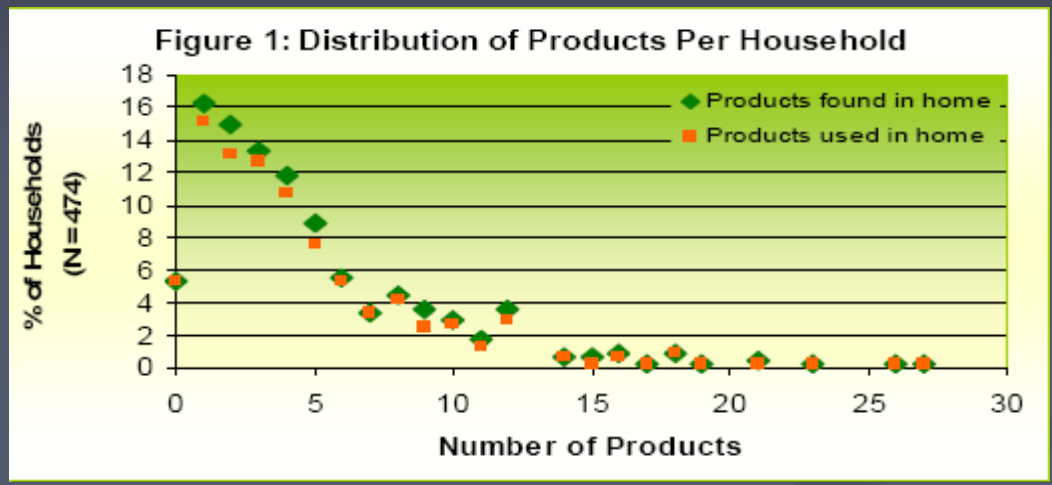
~ 450 households enrolled (2001-2006)

~ 2,300 products inventoried

~ 90% of products *s*uccessful linked to the EPA databases

Source: Guha et al. ISEE Conference, Mexico City, 2007 (Abstract)

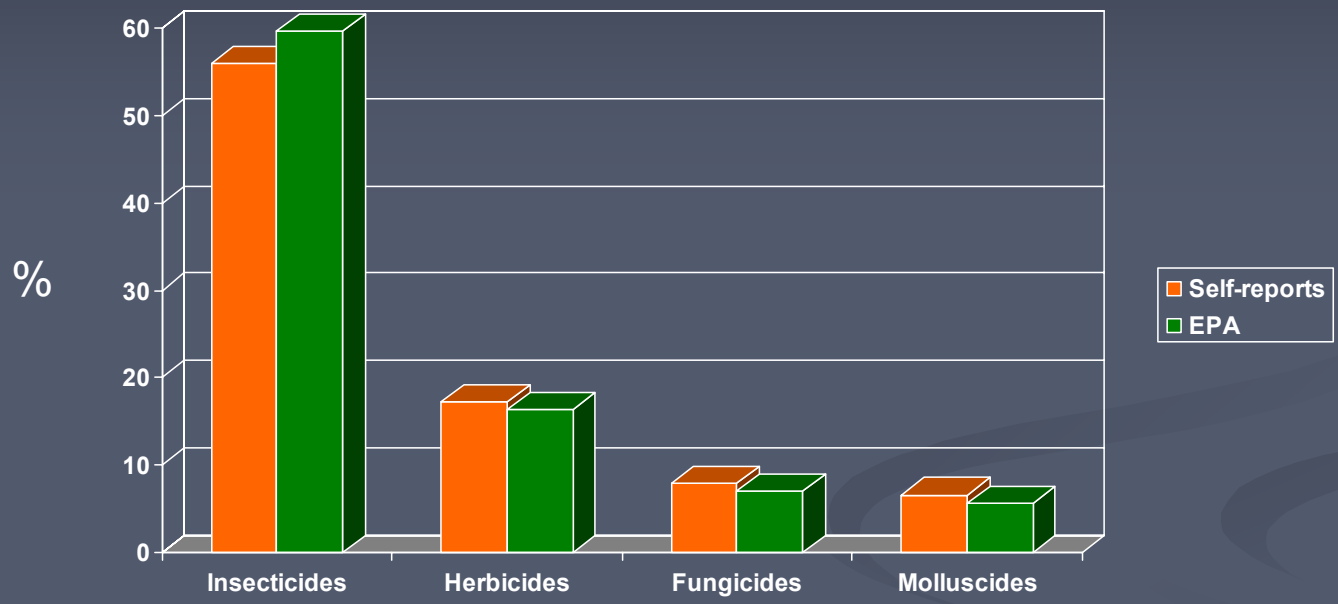
Number of Pesticides



More products found in households of

- controls ($p = 0.01$)
- non-Hispanic whites ($p < 0.0001$)
- higher SES ($p < 0.0001$)

Pesticides by Target Pest and Time Window



- 72% of households used any inventoried pesticides within the last 12 months
- 45% after the child's birth
- 32% during pregnancy
- 8% during the 3 months prior to pregnancy

10 Most Common Active Ingredients

				CARCINOGENICITY	
PREVALENCE (%)	**ACTIVE INGREDIENT**	**PESTICIDE USE**	**CHEMICAL CLASS**	**EPA**	**IARC**
7.5	Pyrethrum Extract	Insecticide	Botanical	Suggestive	Not listed
7.1	Pybuthrin	Synergist	Unclassified	C, Possible	3, Unclassifiable
6.1	Permethrin	Insecticide	Pyrethroid	Suggestive	3, Unclassifiable
5.2	Isopropylamine glyphosate	Herbicide	Phosphonoglycine	Not listed	Not listed
3.2	2,4,6,8-Tetramethyl-1,3,5,7-tetroxocane	Molluscicide	Aldehyde	Suggestive	Not listed
3.1	Cypermethrin	Insecticide	Pyrethroid	C, Possible	Not listed
2.9	Imiprothrin	Not listed	Unclassified	Not listed	Not listed
2.8	Phthalthrin, Tetramethrin	Insecticide	Pyrethroid	C, Possible	Not listed
2.8	N-Octyl bicycloheptene dicarboximide	Synergist	Dicarboximide	C, Possible	Not listed
2.8	Esbiothrin	Fungicide	Morpholine	Not likely	Not listed

C, Possible (limited evidence of carcinogenicity in animals, in the absence of human data, 1996 classification)

Suggestive (evidence from animal and human data that is suggestive, but judged not sufficient for a conclusion as to human carcinogenic potential, 1999 classification)

Source: Guha et al. ISEE Conference, Mexico City, 2007 (Abstract)

Active Ingredients

- Our data are consistent with a previous publication by Colt et al. (2007)
  - developed a matrix of individual active ingredients in home and garden pesticides used in past decades.
  - showed that pyrethrins and permethrins would be the most prevalent active ingredients.

Preliminary Findings

- Interviews and reproducibility
- Pesticide inventory
- ✓ Home dust sampling

Rationale for Dust Sampling

- Children are exposed to carpet dust directly
- Reservoir of pesticides from multiple sources
  - indoor or lawn/garden use
  - “take home” from parental workplaces
  - drift from nearby agricultural areas
- Other compounds of interest and less studied
  - PAHs (combustion sources)
  - PCBs (urban industrial areas)
  - Metals

NCCLS Home Sample Collection

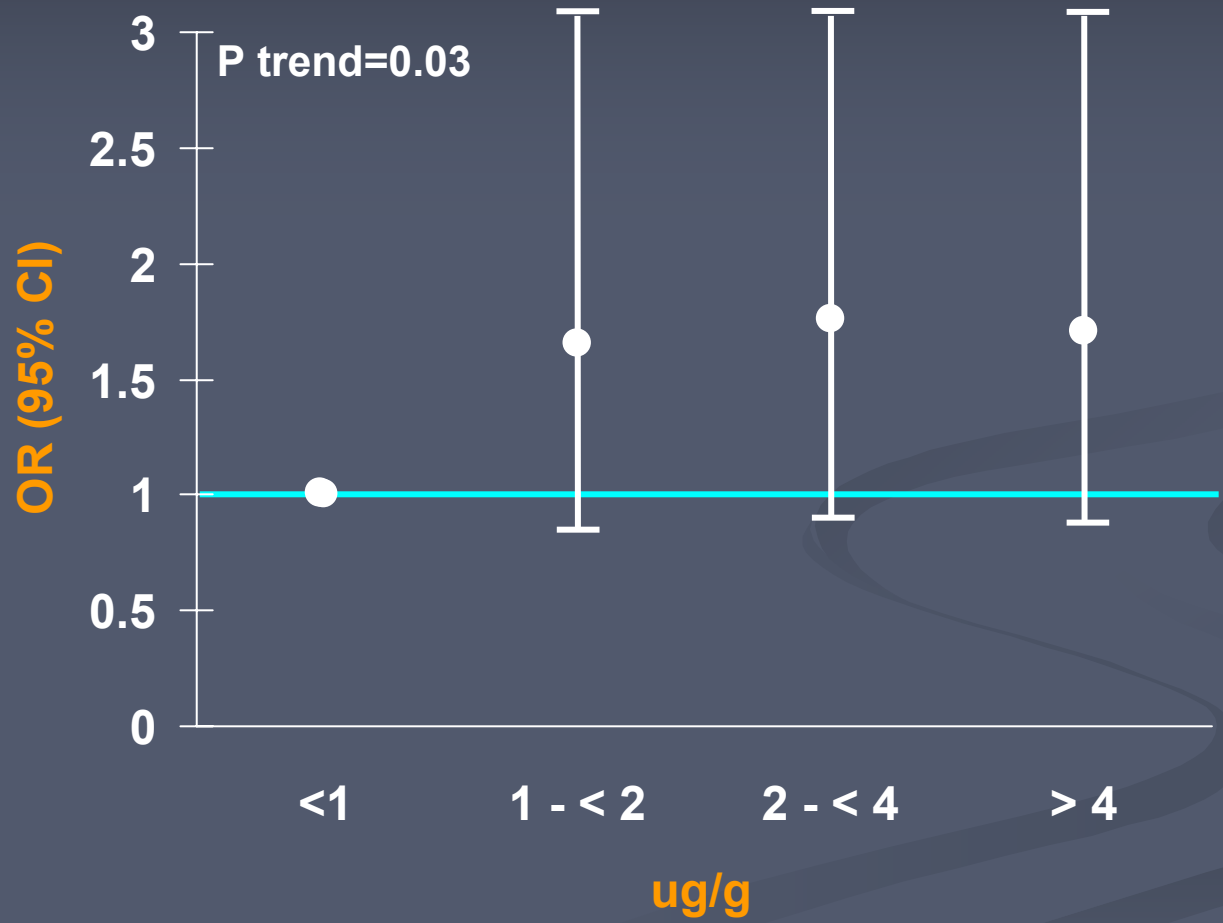
Collaboration with the US National Cancer Institute

- 2nd home visit 3-12 months after 1st home interview (2001-2007)
- Inclusion criteria:
  - child's age at diagnosis <8 years old and corresponding reference date for controls
  - Same residence as diagnosis/reference date
- 90% of eligible families participated in the home environment sampling
  - Carper dust
  - Window sill
  - Air monitors

NCCLS Dust Sample Collection

- ~85% with carpet dust samples
  - Specialized vacuum
  - Used vacuum bag
- Laboratory
  - 42 pesticides
  - 9 PAHs
  - 6 PCBs
  - 9 metals

Childhood ALL and Dust Concentration of Herbicide Dacthal (206 cases - 211 controls)

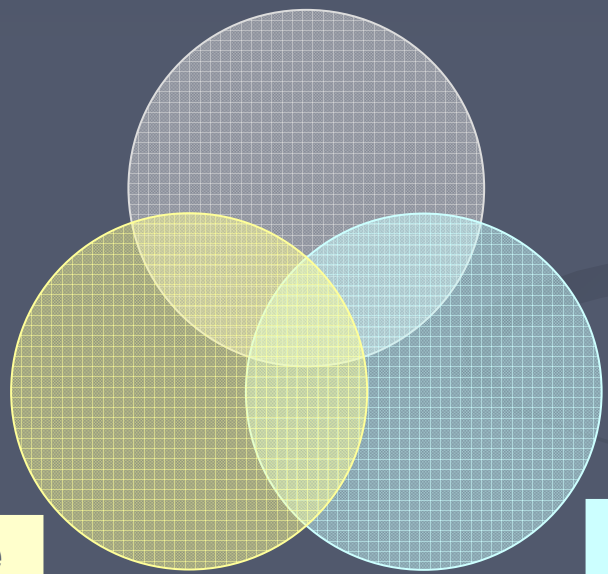


Adjusted for age, gender, race, Hispanic ethnicity, income, interview date

Source: NCCLS personal communication

Method Studies Underway

Interviews – all subjects
Pesticides at home
Parental occupations
Residential history



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Home Dust Sampling

- An important tool to provide objective measures.
- Caveat: current measure to characterize past exposures.
  - Need for repeated samples to assess sampling and seasonal variation.
  - Need to replicate in independent series
- Feasibility
  - Collection of vacuum bags provides similar results than HVS3 (Colt et al., 2007)

Collaborators

National Cancer Institute:

Mary Ward, Joanne Colt, Matt Airola

CDC, Division of Laboratory Sciences,

John Osterloh, Ben Blount

Northern California Cancer Center

Peggy Reynolds, Bob Gunier, Rudy Rull, Julie von Behren

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Sponsors

- National Institute of Environmental Health Sciences (NIEHS)
- National Cancer Institute (NCI)
- Children with Leukaemia (CwL), UK
- Center for Disease Control and Prevention