National Cancer Institute

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OCTOBER 11, 2011, BIOMARKERS IN PESTICIDE, SURVEILLANCE, AND EPIDEMIOLOGY RESEARCH

21ST CENTURY TOXICOLOGY STATE HOLDER WORKSHOP.

Agenda

1) Introduction
   - Background and design of the AHS

2) Cancer epidemiology/biomarker studies
   - e.g., multiple myeloma, prostate cancer

3) Exposure assessment in the AHS

1. Introduction

- **1992** – Concept for the Agricultural Health Study presented to NCI peer review
- **1993** – Field Work Began December 13, 1993
- **1993** – Cancer Incidence and Mortality Monitoring: 1993-2008

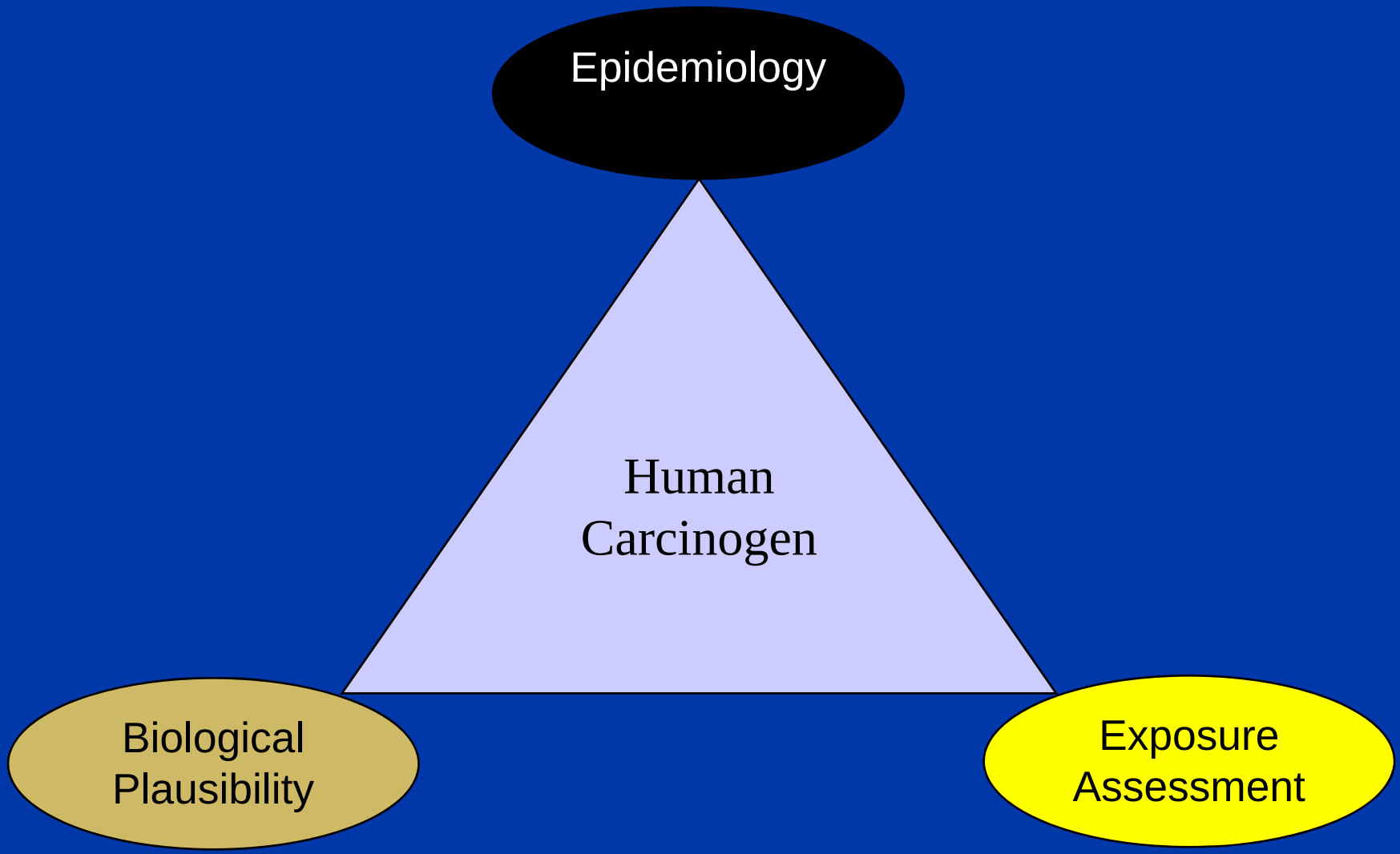
Goal of Research Program:

- Identify human carcinogens among 80 active ingredients in pesticide formulations used occupationally by hundreds of million people world-wide.

Background

- Only 1 pesticide (arsenical insecticides) and 1 pesticide contaminant (dioxin) are classified as Human Carcinogens by IARC, although many others are suspected carcinogens.
- Previous health studies characterized as having inadequate exposure assessment, reducing our ability to identify agents responsible for disease (Zahm et al., 1997, Kromhout and Heedrick 2005).
- Case-control studies (case-recall bias)
- Factory-based pesticide studies—frequently too small to assess individual pesticides for cancer.

Causal Logic to Establish Human Carcinogenicity



Agricultural Health Study (AHS) Design

- Prospective (exposures assessed prior to cancer onset):
  - 52,000 private applicators (i.e., farmers)
  - 32,000 spouses of farmers
  - 5,000 commercial applicators

- Two important agricultural states (Iowa & North Carolina)
  - Corn, soybean and hog production in both states
  - Distinctive agriculture in North Carolina: fruits, vegetables, tobacco, cotton

_AHS Design

- Little loss to cancer incidence follow-up (<2 %)
  - Population-based cancer registries in both states
  - Determine if study subjects move from state (IRS records)
  - National Death Index (NDI)-no loss to mortality follow-up
- Over one-million person-years of follow-up

2. Cancer Epidemiology/Biomarker Studies

2002
- AHS Exposure Algorithm (Dosemeci et al., Annals of Ind. Hygiene; 46:245-260)

2003
- First Nested Case-Control Study: Prostate Cancer (Alavanja et al., AJE; 157:800-814)

2004
- First Cohort Analysis: Alachlor (Lee W et al., AJE; 159:378-830)

Goals of Research Program:

- Identify human carcinogens among 80 active ingredients in pesticide formulations used occupationally by hundreds of millions of people world-wide.
  - Establish Exposure Algorithm
  - Nested Case-Control Analyses (N=5)
  - Follow-up Cohort Analyses (N=27)
  - Biomarker Studies

Multiple Myeloma (MM)

2005
- MM SIR= 1.34 (0.97-1.81) (Alavanja et al., Scand J Work Environ; 31;39-45)

2009
- MGUS 2-fold excess in AHS (Landgren et al., Blood; 113: 6386-6391)

2010
- Initiated Biomarker Study (BEEA) (Alavanja et al.)

Specific Aims:

- Identify pesticides that may be responsible for the excess MM risk in the AHS.
- Identify pesticides and other occupational exposures etiologically linked to monoclonal gammopathy of undetermined significance (MGUS), a confirmed precursor of MM.

Multiple Myeloma (MM)

- **A** largely incurable neoplasm of plasma cells characterized by an overproduction of monoclonal immunoglobulins
- Etiology not well understood, occurs in excess among farmers (Milham S, Am J Epidem 1971, 94(4):507-510)
- MM is highly fatal

MGUS Precedes Multiple Myeloma (MM): in a Prospective Study (PLCO)

- MGUS ----> MM
  - MM always preceded by a premalignant disorder MGUS [monoclonal gammopathy of undetermined significance]. (Landgren O et al., Blood 2009;113:5412-5417)

Risk of MGUS in AHS vs. Olmstead County, MN

Population	Total, n	MGUS, n	OR (95% CI)
Olmstead County	9,469	350	1.0 (ref)
AHS cohort	555	38	1.9 (1.3-2.7)

-Landgren O et al., Blood (2009); 113(25):6386-6391
-Protein Immunology Laboratory at Mayo Clinic, Rochester, Minnesota
(Robert Kyle, Jerry Katzmann, Vincent Rajkumar)

Specific Pesticide Use at Enrollment and Risk of MGUS in 2008 Among 679 Male Applicators in the AHS

Pesticide	Exposed	Total n	Exposed n	OR (95% CI)
Dieldrin	Never	649	31	1.0 (ref)
	Ever	20	6	5.6 (1.9-16.6)
Carbon tetrachloride/ Carbon disulfide mix	Never	632	31	1.0 (ref)
	Ever	41	7	3.9 (1.5-10.0)
Chlorothalonil	Never	649	31	1.0 (ref)
	Ever	20	6	2.4 (1.1-5.3)

-Landgren O et al., Blood (2009); 113(25):6386-6391
-Protein Immunology Laboratory at Mayo Clinic, Rochester, Minnesota (Robert Kyle, Jerry Katzmann, Vincent Rajkumar)

Permethrin Use at Enrollment and Risk of Multiple Myeloma in the AHS

Tertile	Intensity-Weighted Lifetime Exposure-Days			
	No	RR	(95% CI)	*p*-trend
0	29	1.0 (ref.)		
1	2	0.92	(0.22-3.85)	
2	3	1.55	(0.47-5.12)	
3	10	5.01	(2.41-10.42)	<0.01

-Rusiecki et al., Environ Health Perspect (2009); 117(4):581-586

Current Research for MM

- Initiated the Biomarkers of Exposure and Effect in Agriculture study (Alavanja et al., BEEA Study, 2010)
  - 1,600 AHS study subjects will donate blood and urine samples (2010-2014)
  - Biomarker questionnaire to assess current exposures

Potential Future Work on BEEA: Other Biomarkers of Potential Interest

- Measure monoclonal B-cell lymphocytosis (MBL)
- Measures of oxidative stress
- Measures of epigenetic changes
- Markers of immune perturbation
- Chromosomal aberrations
- Other biomarkers as appropriate

Prostate Cancer

2003
- Nested Case-Control (Alavanja et al., AJE; 157:800-814)

2006
- Initiated G X E Study
- iSelect Platform; NCI Core Genotyping Facility

2010
- 8q24 Interaction with Fonofos (Koutros et al., Can Res. 70:9221-9233)
- Other Pathways

Specific aims:

- Identify pesticide exposures that may be responsible for the excess prostate cancer risk in the AHS cohort.
- Identify markers of susceptibility that may be associated with prostate cancer etiology in the AHS cohort.

Nested Case-Control Study

(Alavanja et al., AJE 2003, 157:800-814)

Prostate cancer risk (Significant interaction with family history PC):

- Fonofos
- Coumaphos
- Phorate
- Permethrin
- Butylate

- Terbufos (Near significant interaction with family history of PC)

Risk of Prostate Cancer by Fonofos Exposure With and Without [a] Family History of Prostate Cancer in the AHS

Pesticide	PC risk, no family history of PC			PC risk, family history of PC			Statistical interaction, PC history & Pesticide Exposure
Lifetime exposure days	Odds Ratio	95% C.I.	Cases	Odds Ratio	95% C.I.	Cases	
0	1.00	Ref.	534	1.00	Ref.	100	
>0-20	1.08	0.82-1.41	58	1.42	0.84-2.41	16	1.28 (1.07-1.54)
>20-56	0.93	0.70-1.35	51	1.57	0.95-2.60	18	
>56	0.86	0.60-1.24	30	1.77	1.03-3.05	15	
P trend	P=0.37			P=0.02			

Mahajan R et al. Environ Health Perspecti (2006); 114 (12): 1838-1842

Case-Control Study of Prostate Cancer; Gene-by-Environment Interaction

Chromosome 8q24 , fonofos exposure and prostate cancer risk			
	No fonofos exposure	Low fonofos exposure	High fonofos exposure
Odds Ratio	1.17	1.30	4.46
95% C.I.	0.93-1.48	0.75-2.27	2.17-9.17

- Koutros, et al., Cancer Research 2010; 70(22):9224-9233
-previously identified variant rs4242382
-adjusted *P*-interaction=0.02
-776 cases + 1,444 controls

Case-Control Study of Prostate Cancer; Gene-by-Environment Interaction (continued)

Chromosome 8q24 , terbufos exposure and prostate cancer risk			
	No terbufos exposure	Low terbufos exposure	High terbufos exposure
Odds Ratio	1.13	1.71	2.15
95% C.I.	0.87-1.47	1.07-2.74	1.32-3.52

-Koutros, et al., Cancer Research 2010; 70(22):9224-9233
-previously identified variant rs4242382
-adjusted *P*-interaction=0.02
-similar effect modification for fonofos, coumaphos, phorate, permethrin
-fonofos, phorate and terbufos are phosphorodithioates

Future/Current Biomarker work in this Case- Control Study

- Susceptibility genes (*replication necessary*):
  - Base-excision repair (BER)- Hughes Barry et al.
  - Nucleotide excision repair (NER)- Hughes Barry et al.
  - Xenobiotic metabolizing enzymes (XME)-Koutros et al.
  - Others genes/pathways from prostate etiology literature
- Telomere length- Hou, et al., ongoing
- Epigenetics- Hou,et al., ongoing

3. Exposure Assessment in AHS

2002
- AHS Exposure Algorithm (Dosemeci et al., Annals of Ind Hygiene; 46:245-260).

2010
- Assessment of Algorithm (Thomas et al., J Exp Sci Env Epidemiol; 20:193-134)

2010
- Assessment of Algorithm (Coble et al., Submitted)

Specific aims:

- Optimize questionnaire-based exposure assessment by improving the exposure algorithm

Intensity Weighted Exposure Days

Intensity Weighted Exposure Days=

Total Days of Specific Pesticide Use X Intensity Score

AHS Exposure Assessment Algorithm

Intensity Score=

(Mix + Application Method + Repair) * PPE

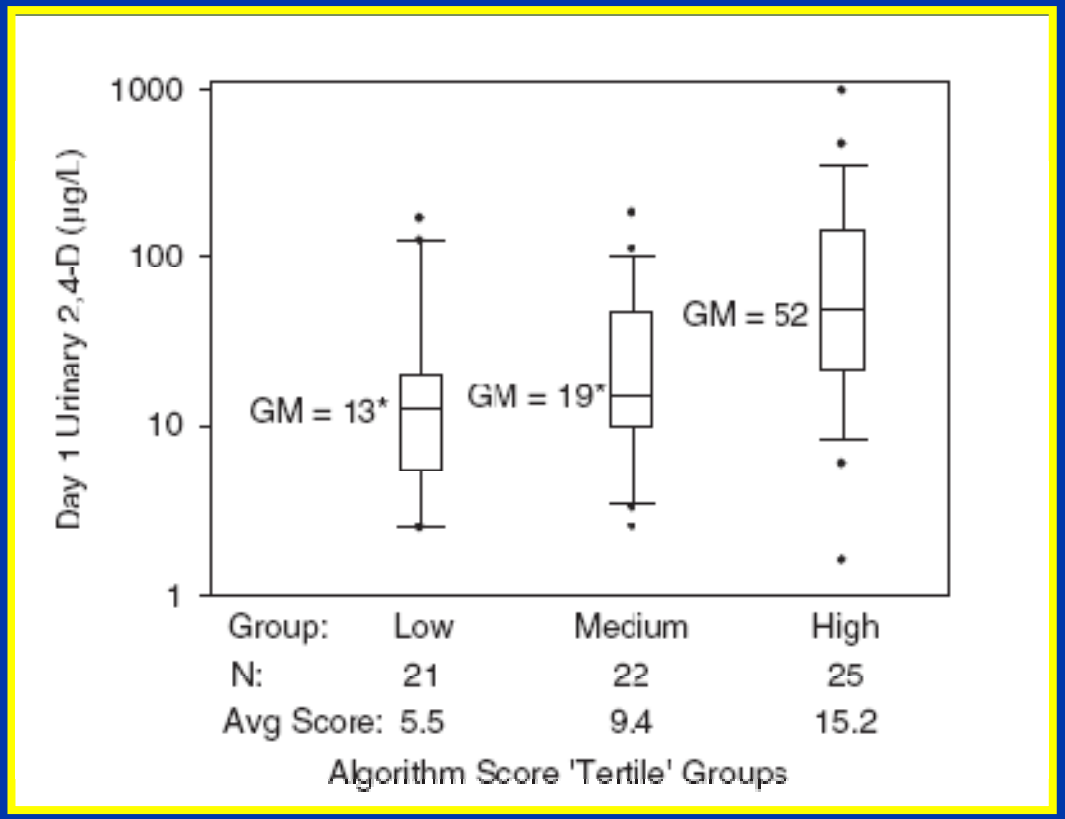
Dosemeci M et al. (2002) Ann Occup Hyg; l 46 (2); 245-260.

AHS Algorithm Intensity Score Evaluation

Algorithm intensity scores from observations and an interviewer administered questionnaire and correlation between scores

	Observation		Questionnaire		Spearman	
	Mean ±SD	Range	Mean ±SD	Range	r	P-value
2,4-D	9.9 ±4.5	1.8-20	10.3 ±4.6	3.0-20.0	0.92	<0.001
Chlorpyrifos	9.2 ± 2.4	4.4-14	9.4 ±2.6	6.6-14	0.84	<0.001

Thomas et al.; J Exposure Science and Environ Epidem; 2010;20(6):559-569



Distributions of Day-1 post-application urinary 2,4-D concentrations across three "tertiles" of algorithm intensity scores (*geometric mean [GM] values for low and medium groups are significantly different from the GM in the high group).

Future Cancer Etiology in AHS

- Reevaluate approximately 30 pesticides for various cancers- e.g., atrazine-Beane Freeman et al.
- Evaluate less frequently used pesticides in AHS
- Evaluate cancers of lower frequency (e.g., leukemia, NHL)
- Biomarkers of Exposure and Effect in Agriculture (BEEA)
  - MGUS
  - MBL
  - Other
  - Expand the Study
- G X E Analysis for Prostate Cancer
- Environmental Cancer Risk —(e.g., Dr. Ward: drinking water, Dr. Beane Freeman: endotoxin)

Intramural Research Team

- National Cancer Institute:
  - Michael Alavanja Co-PI
  - Laura Beane Freeman Co-PI
  - Mary Ward
  - Sonja Berndt
  - Stella Koutros
  - Gabriella Andreotti
  - Jonathan Hofmann
  - Neil Caporaso
  - Ola Lundgren
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  - Rashmi Sinha
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Thank you.

- **Questions?**