Interpretation of Epidemiologic Studies

Paolo Boffetta
Mount Sinai School of Medicine, New York, USA
International Prevention Research Institute, Lyon, France
Outline

• Introduction to epidemiology
• Issues in epidemiologic research
  • weak associations
  • false positive results
   • occupational carcinogens
• Pesticides and cancer
• Conclusions
Epidemiology

• Study of health-related conditions in human populations
  • diseases, characteristics, interventions

• Aims
  • descriptive
  • etiologic (associations)
Etiologic research in epidemiology

- Comparison of occurrence of disease in populations with different patterns of exposure to the agent under investigation
  - (i) retrospective assessment of exposure in a group of patients (cases) and a comparable group of controls
  - (ii) prospective investigation of disease occurrence in a group of exposed subjects and a comparable group of unexposed

- Statistical estimate of the ‘association’ between exposure and disease
  - risk of disease in exposed relative to unexposed
Key assumption

• Except for disease (i) or exposure (ii), all other characteristics are similar* between the groups being compared
  • this is fully achieved only in randomized trials
  • under some circumstances, other characteristics can be ‘adjusted for’ in the statistical analysis

* similar with respect to exposure (i) or disease (ii)
Causality in epidemiology

• Epidemiology leads to the identification of associations between risk factors and diseases at the population level

• Observational nature of most epidemiological investigations
  – potential role of systematic error (bias and confounding)

• Causal inference in epidemiology requires systematic error to be excluded
  – in addition to exclusion of random error, consistency of results across studies and coherence with other lines of evidence
Hill’s ‘criteria’ for causality

- In 1965 Hill proposed a set of guidelines, derived from those used in a 1964 US SG Report on Health Effects of Smoking to establish the causal nature of the association between tobacco smoking and lung cancer.
- Hill’s guidelines have become the paradigm of criteria to evaluate the causal nature of results of observational studies – several modifications have been proposed since.
Hill’s ‘criteria’ for causality

- Strength of association
- Temporality
- Consistency
- Theoretical plausibility
- Coherence
- Specificity in the causes
- Dose response relationship
- Experimental evidence
- Analogy
Strength of association

• The association between a risk (or protective factor) and cancer is measured by comparing the occurrence of cancer in groups defined according to exposure to the risk factor – ratio of disease risk/odds/rate in exposed and unexposed.

• The stronger the relationship between the independent variable and the dependent variable, the less likely it is that the relationship is due to an extraneous variable.
Risk ratio in an hypothetical cohort study (1/2)

<table>
<thead>
<tr>
<th></th>
<th>Exposed</th>
<th>Non-exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>10,000</td>
<td>10,000</td>
</tr>
<tr>
<td>Cases</td>
<td>1,500</td>
<td>100</td>
</tr>
<tr>
<td>Non-cases</td>
<td>8,500</td>
<td>9,900</td>
</tr>
</tbody>
</table>

Risk ratio $0.15/0.01 = 15$
95% confidence interval 12.3, 18.3
Risk ratio in an hypothetical cohort study (2/2)

<table>
<thead>
<tr>
<th></th>
<th>Exposed</th>
<th>Non-exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>10,000</td>
<td>10,000</td>
</tr>
<tr>
<td>Cases</td>
<td>120</td>
<td>100</td>
</tr>
<tr>
<td>Non-cases</td>
<td>9,880</td>
<td>9,900</td>
</tr>
</tbody>
</table>

Relative risk $0.012/0.01 = 1.2$
95% confidence interval 0.92, 1.56
Weak associations

- Intuitively, we lend more credibility to "strong" than to "weak" associations – where is the boundary?
- Weak associations are more likely to be explained by chance, bias and confounding (and their combinations)
## Examples of ‘old’ carcinogens

<table>
<thead>
<tr>
<th>Agent</th>
<th>Target organ</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunlight</td>
<td>Skin</td>
<td>3</td>
</tr>
<tr>
<td>Tobacco chewing</td>
<td>Oral cavity</td>
<td>4</td>
</tr>
<tr>
<td>Tobacco smoking</td>
<td>Lung</td>
<td>15</td>
</tr>
<tr>
<td>Alcohol drinking</td>
<td>Oral cavity</td>
<td>5</td>
</tr>
<tr>
<td>Aromatic amines</td>
<td>Bladder</td>
<td>8</td>
</tr>
<tr>
<td>Asbestos</td>
<td>Lung</td>
<td>5</td>
</tr>
</tbody>
</table>

WHO, 1964
Examples of ‘new’ carcinogens

<table>
<thead>
<tr>
<th>Agent</th>
<th>Target organ</th>
<th>Year</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobacco smoking</td>
<td>Liver</td>
<td>2004</td>
<td>1.6</td>
</tr>
<tr>
<td>Involuntary smk</td>
<td>Lung</td>
<td>2004</td>
<td>1.25</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>NPC</td>
<td>2007</td>
<td>1.3</td>
</tr>
<tr>
<td>Alcohol drinking</td>
<td>Breast</td>
<td>2007</td>
<td>~1.2</td>
</tr>
<tr>
<td>1,3 Butadiene</td>
<td>Lymphohem.</td>
<td>2008</td>
<td>1.15</td>
</tr>
</tbody>
</table>
False positive results

- False positive results are an inherent feature of biomedical research
- The probability that a ‘positive’ result is true depends on
  - prior probability of the result being true
  - statistical power of the study
  - level of statistical significance
- Results of small studies investigating weak determinants with low prior probability are particularly prone to be false
  - the majority of positive research findings, especially those based on large-throughput molecular approaches involving many “determinants” and “outcomes”, are likely to be false
Chance vs. bias

- False positive results are generated by chance and bias
  - False positive results generated by chance are hardly replicated in subsequent investigations
  - Bias bias may operate in a similar fashion in different settings and populations, thus providing a consistent pattern of independently generated positive results

- The problem is exacerbated by the tendency in many epidemiological studies to generate large sets of results and to selectively report ‘positive’ or ‘significant’ findings
Avoiding false positive results in epidemiology

• Generation of wrong scientific evidence, leading to wrong societal and public health decisions
  – ex. coffee drinking and risk of pancreatic cancer
• Loss of credibility
• Waste of public resources
• False positive results generated by chance will eventually be corrected by replication
  – lengthy, costly and inefficient practice
False positive results in epidemiology

- Several areas of observational epidemiology are particularly prone to false positive findings
  - weak associations
  - multiplicity of determinants and outcomes
  - subgroup analyses

- Examples from genetic epidemiology
  - review of 36 genetic disease associations (Ioannidis et al., 2001)
    - 25 of them the first report gave a stronger estimate of protection or predisposition than subsequent research (for 10 p<0.05)
  - review of 55 meta-analyses, including 579 study comparisons, of genetic associations (Ioannidis et al., 2003)
    - for each association the largest study generally yielded more conservative results than the meta-analysis
    - in 26 percent of the meta-analysis the association was significantly stronger in the first study
    - only in 16% the genetic association found in the first study was replicated without evidence of heterogeneity and bias

- Statistical tools are available
  - false discovery rate
  - false positive report probability
  - Bayesian false-discovery probability
False positive results in occupational cancer epidemiology

• Assessment of whether early results on suspected occupational carcinogens were more positive than subsequent results
  – based on the evaluations made within the IARC Monographs program

• Inclusions
  – occupational agents for which the evidence of carcinogenicity in humans was classified as ‘limited’ or ‘inadequate’
  – studies available at the time of the most recent Monographs evaluation
  – agents for which 3 or more epidemiological studies were available
Results

- 23 associations
  - 150 risk estimates
- Changes in risk estimates over time
  - decrease in 12
  - no change in 6
  - increase in 5
Acrylonitrile and lung cancer – cumulative meta-analysis
(16 studies; 314 cases)

Boffetta et al., 2008
Welding fumes and lung cancer – cumulative meta-analysis (11 studies; 407 cases)
Caveats

- No detailed review and evaluation of the evidence linking each agent to each cancer
- Restriction to historical cohort studies
- Exclusion of internal (e.g., dose-response) analyses available in some cohorts
- Bias and confounding not addressed for each study
- Restriction to data available at the time of the most recent IARC Monograph
First nested case-control study of serum DDE and breast cancer risk

Wolff et al, 1993
Cumulative meta-analysis of studies of serum DDE and breast cancer risk
IARC Monographs Evaluations

- Human data
  clinical studies
  epidemiologic studies
- Animal data
  long-term studies
- Mechanistic data
  genotoxicity, etc.
IARC Evaluations

- Human data
  clinical studies
  epidemiologic studies
- Animal data
  long-term studies
- Mechanistic data
  genotoxicity, etc.

Group 1
Established carcinogen
IARC Evaluations

- Human data
  - clinical studies
  - epidemiologic studies
- Animal data
  - long-term studies
- Mechanistic data
  - genotoxicity, etc.

Group 1
Established carcinogen
IARC Evaluations

- Human data
  - clinical studies
  - epidemiologic studies
- Animal data
  - long-term studies
- Mechanistic data
  - genotoxicity, etc.

Group 2A
Probable carcinogen
IARC Evaluations

- Human data
  clinical studies
  epidemiologic studies
- Animal data
  long-term studies
- Mechanistic data
  genotoxicity, etc.

Group 2B
Possible carcinogen
Pesticides classified in Group 1

• Arsenic
• Ethylene oxide
• TCDD*

* contaminant
<table>
<thead>
<tr>
<th>Agent</th>
<th>YLE</th>
<th>Hum</th>
<th>An</th>
</tr>
</thead>
<tbody>
<tr>
<td>Application of insectides</td>
<td>1991</td>
<td>L</td>
<td>-</td>
</tr>
<tr>
<td>Captafol</td>
<td>1991</td>
<td>ND</td>
<td>S</td>
</tr>
<tr>
<td>Ethylene dibromide</td>
<td>1999</td>
<td>I</td>
<td>S</td>
</tr>
</tbody>
</table>
Selected pesticides classified in group 2B (N=19)

<table>
<thead>
<tr>
<th>Agent</th>
<th>YLE</th>
<th>Hum</th>
<th>An</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clordane</td>
<td>2001</td>
<td>I</td>
<td>S</td>
</tr>
<tr>
<td>Chlordecone</td>
<td>1987</td>
<td>ND</td>
<td>S</td>
</tr>
<tr>
<td>Chlorophenoxy herbicides</td>
<td>1987</td>
<td>L</td>
<td>I</td>
</tr>
<tr>
<td>DDT</td>
<td>1991</td>
<td>I</td>
<td>S</td>
</tr>
<tr>
<td>Heptachlor</td>
<td>2001</td>
<td>I</td>
<td>S</td>
</tr>
<tr>
<td>Reference</td>
<td>Population</td>
<td>N</td>
<td>Country</td>
</tr>
<tr>
<td>---------------</td>
<td>-----------------------</td>
<td>-------</td>
<td>----------</td>
</tr>
<tr>
<td>MacMahon 1988</td>
<td>Pest control workers</td>
<td>16124</td>
<td>USA</td>
</tr>
<tr>
<td>Blair 1983</td>
<td>Pest control workers</td>
<td>4411</td>
<td>USA</td>
</tr>
<tr>
<td>Alavanja 1990</td>
<td>Flour millers</td>
<td>22938</td>
<td>USA</td>
</tr>
<tr>
<td>Corrao 1989</td>
<td>Licensed farmers</td>
<td>25945</td>
<td>Italy</td>
</tr>
<tr>
<td>Barthel 1981</td>
<td>Plant protection wrk</td>
<td>1658</td>
<td>Germany</td>
</tr>
<tr>
<td>Wiklund 1989</td>
<td>Licensed applicators</td>
<td>20245</td>
<td>Sweden</td>
</tr>
</tbody>
</table>
Risk of selected cancers among insecticide spayers and applicators

- Lung ca
- LHP
- NHL
- Brain
- Skin

MacMahon  Blair  Alavanja  Corrao  Barthel  Wiklund
International study of pesticide producers and applicators

<table>
<thead>
<tr>
<th>Cancer</th>
<th>N</th>
<th>RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>All neoplasms</td>
<td>1127</td>
<td>1.06</td>
<td>(1.00 - 1.13)</td>
</tr>
<tr>
<td>Lung</td>
<td>380</td>
<td>1.09</td>
<td>(0.98 - 1.20)</td>
</tr>
<tr>
<td>Other respiratory</td>
<td>12</td>
<td>2.25</td>
<td>(1.16 - 3.93)</td>
</tr>
<tr>
<td>Soft-tissue sarcoma</td>
<td>9</td>
<td>2.00</td>
<td>(0.91 - 3.79)</td>
</tr>
<tr>
<td>Breast</td>
<td>12</td>
<td>1.23</td>
<td>(0.63 - 2.14)</td>
</tr>
<tr>
<td>Endometrium</td>
<td>4</td>
<td>2.30</td>
<td>(0.63 - 5.89)</td>
</tr>
<tr>
<td>Non-Hodgkin l.</td>
<td>34</td>
<td>1.27</td>
<td>(0.88 - 1.78)</td>
</tr>
<tr>
<td>Hodgkin l.</td>
<td>10</td>
<td>0.99</td>
<td>(0.48 - 1.82)</td>
</tr>
</tbody>
</table>

Kogevinas et al., 1997
Risk of NHL among farmers

- Meta-analysis of 36 studies [Khuder et al., 1998]
  - overall RR 1.10 (95% CI 1.03-1.19)
  - higher for men, US studies and case-control studies
  - strong heterogeneity
## Risk of NHL among farmers

Studies published after 1997

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>RR</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mester 2006</td>
<td>Germany</td>
<td>2.4</td>
<td>1.1-5.4</td>
</tr>
<tr>
<td>Lee 2002 - crop</td>
<td>USA</td>
<td>1.01</td>
<td>0.96-1.06</td>
</tr>
<tr>
<td>- livestock</td>
<td></td>
<td>1.17</td>
<td>1.06-1.30</td>
</tr>
<tr>
<td>McDuffie 2002</td>
<td>Canada</td>
<td>1.53</td>
<td>1.04-2.25</td>
</tr>
<tr>
<td>Zheng 2002</td>
<td>USA</td>
<td>2.0</td>
<td>1.5-2.8</td>
</tr>
<tr>
<td>Costantini 2001</td>
<td>Italy</td>
<td>0.8</td>
<td>0.6-1.1</td>
</tr>
<tr>
<td>Settimi 1999</td>
<td>Italy</td>
<td>1.0</td>
<td>0.5-2.3</td>
</tr>
<tr>
<td>Epilymph unpubl.</td>
<td>Europe</td>
<td>1.02</td>
<td>0.88-1.19</td>
</tr>
<tr>
<td>Meta-analysis</td>
<td></td>
<td>1.05</td>
<td>1.01-1.10</td>
</tr>
</tbody>
</table>
Issues in the interpretation of epidemiological studies on pesticides and cancer

• **Chance**
  – study too small to detect a weak effect
  – positive associations resulting from multiple comparisons

• **Confounding**
  – another risk factor is responsible for the observed effect

• **Bias**
  – lack of comparability of exposed and unexposed
  – different recall
  – publication bias
Publication bias

- It originates from the tendency of authors and journal reviewers and editors to report and publish ‘positive’ or ‘statistically significant’ results over ‘null’ or ‘non-significant’ results.
- It might generate a false consistency among studies
- Statistical tests have been proposed to assess the presence of publication bias
  - low power
  - need for a large number of independent studies
- Example of 2,3,7,8-TCDD and NHL
Studies of dioxin exposure and NHL risk

Meta-analysis RR 1.6; 95% CI 1.2-2.1
Begg’s test for publication bias: p=0.02
Conclusions

• Weak associations are a major challenge to epidemiologic research
• Need to reduce likelihood of false positive results
  – adherence to the highest epidemiological standards in the design, analysis, reporting and interpretation of studies
• Pesticides and cancer
  – few agents are known or suspected carcinogens
  • strong regulation for most of them
  – current evidence does not suggest a role of pesticide exposure in human cancer