Benzene Health Effects Research: What’s New?

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Topics: Benzene Health Risk Update

• Background – benzene health risks and regulation
• Unanswered questions regarding benzene’s health risks
• Results from recent studies on benzene
• What is myelodysplastic syndrome (MDS)?
• Do we know how benzene produces toxicity/health effects?
• Impact on benzene occupational exposure limits
  • 0.5 ppm TWA
  • 2.5 ppm STEL

• Are there subpopulations at higher risk?
• Are there medical monitoring tests besides the CBC on the horizon?
Background: Benzene Health Effects

• Benzene (BZ) recognized as a human leukemogen (i.e. causes leukemia)
  • acute myeloid leukemia (AML) – definite link to benzene
  • other cell types – designated as “probable” by (IARC, 2010) – debated by others
    • non-Hodgkin lymphoma (NHL)
    • acute and chronic lymphoid leukemia (ALL and CLL)
    • multiple myeloma
  • no mention of myelodysplastic syndrome (MDS) by IARC … little research
  • no mention of other myeloid tumors (myeloproliferative disease)
  • insufficient evidence for chronic myeloid leukemia (CML)

• Benzene has long been known to be a bone marrow toxin and causes hematologic (blood) effects at sufficiently high exposures
  • some (but not all) reports show effects (usually mild, possibly reversible) at low (< 1 ppm) levels

• Benzene is clastogenic (causes chromosomal effects)
  • genotoxicity results in ‘no safe level’ policy for many regulatory groups
Basis for most benzene regulations

Most BZ risk assessments based on Pliofilm study
- Relatively small study
- **All** leukemia subtypes
- Little empirical data on lower (<10 ppm) exposures
Benzene Regulatory Standards

Most regulatory agencies still use pliofilm data to predict excess disease at lower exposure levels.

- Fifteen total leukemia cases in Pliofilm population (all leukemia sub-types)
  - ACGIH: 0.5 ppm (TWA), 2.5 ppm (STEL)
  - EPA: 0.04 ppb (24 hr/day, 70 year lifetime)
Data Gap for BZ exposures < 10 ppm

Current day exposure zone:
Focus of pooled analysis
Key unanswered questions on benzene

L-H disease subtypes
  new WHO classification scheme aids research efforts

Dose response
  particularly for exposures < 10 ppm
  empirical data – linear or non-linear?

Mechanisms of toxicity
  like chemotherapeutic agents?  role of key enzymes – e.g.
  topoisomerase-II?  role of bone marrow inflammation/auto-immunity?

Early events
  is the complete blood count (CBC) useful?
RESULTS FROM RECENT STUDIES ON BENZENE
Pool data from three previous studies

- **Canada**
  - no consistent dose response, but small study
  - based on 31 LH cancers

- **U.K.**
  - inconsistent dose response by subgroups and exposure metrics
  - based on 90 leukemias

- **Australia**
  - strong dose response, especially for AML
  - based on 79 LH cancers

Before pooling data → update studies

- 60 LH cancers
  - Incl. 5 MDS

- 193 LH cancers
  - Incl. 11 MDS

- 117 LH cancers
  - Incl. 13-MDS

370 LH cancers

References:

1. Schnatter et al., 1996 OEM 53:773-781
2. Rushton et al., 1997 OEM 54:152-166
Lymphohematopoietic (LH) Cancer: Paradigm Shift Recently Occurred

Traditional Paradigm: Anatomy
- LEUKAEMIAS (CA in peripheral blood)
- LYMPHOMAS (CA in lymph system)

New Paradigm: Cell of Origin
- MYELOID tumours
  - Myeloproliferative Disease (MPD)
  - Myelodysplastic Syndrome (MDS)
  - Acute Myeloid Leukemias (AML)
- LYMPHOID tumours
  - B-cells (NHL, CLL, ALL)
  - T-cells

New paradigm: biologically justified affects new benzene science will likely affect future benzene regulations
LH cancer classification (WHO scheme)

- **Myeloid Neoplasms (163)**
  - Myeloproliferative Diseases (58)
    - CIMF chronic myelofibrosis (10)
    - Polycythemia vera (11)
    - NOS (7)
    - Other or unclassifiable MPD (2)
  - MDS/MP Diseases (8)
    - CMML (6)
  - Myelodysplastic Syndromes (29)
    - RA, RA-EB (8)
    - Refractory cytopenia w/ML dysplasia (2)
    - MDS, other (5q-) or unclassifiable (1)
  - Acute Myeloid Leukemias (60)
    - AML with or without maturation (i.e. M1/M2) (5)
    - Acute monocytic & acute myelomonocytic leukemia (M4/M5) (3)
    - Acute erythroid leukemia (i.e. M6) (2)
    - AML w/recurrent cytogenetic abnormalities (2)
  - Acute Leukemias of Ambiguous Lineage (3)

**Included in statistical analysis**
- CML (28)
- MDS (29)
- MPD (30)
- AML (60)
Case-control studies: basic design

- Define disease(s) of interest (cases)

- Match cases to those who DO NOT have disease of interest (controls)
  - Typical matching factors: age, gender, time period

- Determine exposures among both groups (usually back in time)
  - “blinding” is important – no knowledge of case/control status

- Compare exposures between cases and matched controls

- If cases are exposed more frequently, or to higher levels, beyond chance, the exposure could be leading to the disease.
Pooled Analysis Study Procedures

• POOLED existing cases and controls

• Added NEW cases and matched controls (4:1 and 5:1) from updated cohort follow-ups to each study
  • Requested source records (hospital, histology, registry) for all cases
  • Two hematopathologists re-classified all cases based on source records

• CASE COUNTS:

<table>
<thead>
<tr>
<th>Disease</th>
<th>n</th>
<th>IARC status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Myeloid Leukemia (AML)</td>
<td>60</td>
<td>Known</td>
</tr>
<tr>
<td>Chronic Myeloid Leukemia (CML)</td>
<td>28</td>
<td>Insufficient</td>
</tr>
<tr>
<td>Chronic Lymphoid Leukemia (CLL)</td>
<td>80</td>
<td>Probable</td>
</tr>
<tr>
<td>Myelodysplastic Syndrome (MDS)</td>
<td>29</td>
<td>Not mentioned</td>
</tr>
<tr>
<td>Myeloproliferative Disease (MPD)</td>
<td>30</td>
<td>Not mentioned</td>
</tr>
</tbody>
</table>

(Pliofilm study: n= 16 “total leukemias”)
Pooled Analysis Study Methods (cont’d)

EXPOSURE ASSESSMENT
• Based on >5800 monitoring results over time
• Calculated six exposure metrics:
  • parts per million x years exposed (ppm-years)
  • average (ppm)
  • maximum (ppm)
  • duration (years)
  • peak (>3 ppm, 15-60 minutes, weekly)
  • dermal exposure probability (H, M, L, N)

INFORMATION QUALITY
• Each exposure estimate scored for certainty (high, medium, low)
• Each diagnosis scored for certainty (high, medium, low)

STATISTICAL ANALYSIS
• Penalized spline models to allow flexible shape of dose-response curves
• Sensitivity analyses on more certain (higher quality) data
Disease Classification

- Certainty scores indicated a wide range of diagnostic certainty

- Certainty driven by:
  - Specificity of terms in source records
  - Documented diagnostic methods
  - Amount/type of source material
  - Agreement among source records

- Certainty scores:
  - AU > CA > UK
  - CML/CLL > AML > MPD/MDS

- Sensitivity analyses on more certain cases
Reclassification changed some diagnoses

<table>
<thead>
<tr>
<th>ORIGINAL DIAGNOSIS</th>
<th>% unchanged</th>
<th>Revised diagnoses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemia</td>
<td>75%</td>
<td>Chronic Leukemia</td>
</tr>
<tr>
<td>Acute Leukemia</td>
<td>86%</td>
<td>AML</td>
</tr>
<tr>
<td>Chronic Leukemia</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Lymphoid Leukemia</td>
<td>50%</td>
<td>CLL</td>
</tr>
<tr>
<td>Myeloid Leukemia</td>
<td>60%</td>
<td>AML, MDS</td>
</tr>
<tr>
<td>Acute Lymphoid Leukemia</td>
<td>78%</td>
<td>CLL, MPD</td>
</tr>
<tr>
<td>Acute Myeloid Leukemia</td>
<td>90%</td>
<td>MDS, MPD</td>
</tr>
<tr>
<td>Chronic Myeloid Leukemia</td>
<td>94%</td>
<td>MDS</td>
</tr>
<tr>
<td>Chronic Lymphoid Leukemia</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Multiple Myeloma</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>non-Hodgkin Lymphoma</td>
<td>90%</td>
<td>ALL, CLL, Lymph NOC.</td>
</tr>
</tbody>
</table>
Dose Response for AML (spline analysis)

Little evidence for dose-response for AML, which is the leukemia subtype most often linked to benzene exposure.
Dose Response for CML (spline analysis)

No dose response for CML
Dose Response for CLL (spline analysis)

No dose response for CLL
Dose Response for MPD (spline analysis)

No dose response for MPD

Cumulative benzene exposure in ppm-years

All workers (p = 0.49)
More certain diagnoses (p = 0.49)
95% CI (all workers)
**Dose Response for MDS (spline analysis)**

Benzene shows a stronger dose-response for MDS compared to other endpoints.
Results for Peak Exposure (> 3 ppm weekly exposure for 15-60 minutes for at least one year)

Peak exposure associated with MDS, but not other LH cancer subtypes
# Key Analyses for MDS from Pooled Analysis

## Cumulative Exposure (ppm-years)

<table>
<thead>
<tr>
<th>Cum. Exp. (ppm-years)</th>
<th>All</th>
<th>High Dx Certainty</th>
<th>High Exp. Certainty</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;.35</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>.35 – 2.9</td>
<td>1.7</td>
<td>3.5</td>
<td>1.7</td>
</tr>
<tr>
<td>&gt;2.9</td>
<td>4.3*</td>
<td>11.6*</td>
<td>4.3</td>
</tr>
</tbody>
</table>

Trend Test: p=0.01, p=0.004, p=0.10

## Average Exposure (ppm)

<table>
<thead>
<tr>
<th>Avg. Exp. (ppm)</th>
<th>All</th>
<th>High Dx Certainty</th>
<th>High Exp. Certainty</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.16-.08</td>
<td>1.99</td>
<td>2.97</td>
<td>3.66</td>
</tr>
<tr>
<td>.08-.26</td>
<td>1.85</td>
<td>3.24</td>
<td>1.89</td>
</tr>
<tr>
<td>&gt;.26+</td>
<td>3.12</td>
<td>6.10</td>
<td>7.66</td>
</tr>
</tbody>
</table>

Trend Test: p=0.08, p=0.04, p=0.08

## Maximum Exposure (ppm)

<table>
<thead>
<tr>
<th>Max. Exp. (ppm)</th>
<th>All</th>
<th>High Dx Certainty</th>
<th>High Exp. Certainty</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.16-.12</td>
<td>1.46</td>
<td>2.13</td>
<td>1.87</td>
</tr>
<tr>
<td>.12-.41</td>
<td>1.82</td>
<td>5.89</td>
<td>2.83</td>
</tr>
<tr>
<td>&gt;.41</td>
<td>2.81</td>
<td>3.32</td>
<td>8.30</td>
</tr>
</tbody>
</table>

Trend Test: p=0.01, p=0.10, p=0.11

## Duration (years)

<table>
<thead>
<tr>
<th>Duration (years)</th>
<th>All</th>
<th>High Dx Certainty</th>
<th>High Exp. Certainty</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤15.6</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>15.6-28</td>
<td>0.77</td>
<td>0.85</td>
<td>0.57</td>
</tr>
<tr>
<td>&gt;28</td>
<td>1.74</td>
<td>3.34</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Trend Test: p=0.22, p=0.08, p=0.69

## Peak Exposure

<table>
<thead>
<tr>
<th>Peak Exposure</th>
<th>All</th>
<th>High Dx Certainty</th>
<th>High Exp. Certainty</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 3ppm</td>
<td>2.48</td>
<td>6.32*</td>
<td>5.74*</td>
</tr>
</tbody>
</table>

Strongest relationships: cumulative exposure and peak exposure metrics.

When both metrics are considered in a single model, only peak exposure remains statistically significant.
Key Analyses for MDS from Pooled Analysis

Spline analysis is more robust statistical measure
- Tertile analysis point of significance 2.93 not significant in spline analysis
- More robust spline analysis indicate borderline significance between 9 and 75 ppm-yrs
  \[9 \text{ ppm-y} / 25 \text{ y} = 0.4 \text{ ppm LTA (based on study population)}\]
Pooled Analysis MDS Cases / Controls

- MDS cases over-represented (beyond 4-5:1 matching ratio) at higher maximum (>0.7 ppm) benzene concentrations.
Summary: pooled nested case control study of benzene exposure

- These results are believed to represent a real association between benzene and MDS at lower levels than previously reported
  - Higher concentrations of benzene may be necessary to affect AML

- Future studies on benzene should include MDS as a potential outcome and seek to measure benzene exposure, smoking, and individual susceptibility factors as precisely as possible.
Is it deemed necessary to have these two sub-bullets on the slide?
Nachman, Jessica R, 19/09/2014
Impact on Occupational Exposure Limits

• In 2012, the EM Occupational Exposure Limit committee considered the pooled analysis findings and:
  • Retired the Benzene ACGIH TLV (0.5 ppm TWA / 2.5 ppm STEL)
  • Adopted a new OEL (0.5 ppm TWA / 1 ppm STEL)

• Reasons for lowering the STEL from 2.5 to 1 ppm:
  • Indications that peak exposures were important in pooled analysis
  • Supporting information from biologic considerations (PBPK models)

• Reasons for maintaining a TWA of 0.5 ppm:
  • peak exposure metric more robust than intensity metrics in a joint risk model
    • observed intensity effects believed to be linked to peak exposures
  • Excess MDS not seen for more continuous exposures (e.g. refinery workers)
  • 0.5 ppm TWA is presently implemented in a way that does not allow full shift concentrations close to 0.5 ppm
  • In an environment with prevalent episodic (rather than continuous) exposure scenarios, lowering the STEL may be an efficient way to lower overall exposures
What is Myelodysplastic Syndrome (MDS)?

- MDS is a ‘relatively’ new disease (1982- FAB, 2001 and 2008- WHO)
  - Diagnosis requires histopathologic examination of the bone marrow (1970’s ff)
  - Likely misclassified as aplastic anemia, myeloproliferative diseases or other LH in the past
- Formerly known as “pre-leukemia” (also “smoldering leukemia”)
  - Now known that only 20-30% progress to leukemia
- Reportable to US (and other) cancer registries as of 2001 (now technically a “cancer”)

### Myelodysplastic Syndromes
- Refractory anaemia
- Refractory anaemia with ringed sideroblasts
- Refractory cytopenia with multilineage dysplasia
- Refractory anaemia with excess blasts
- Myelodysplastic syndrome associated with isolated del(5q) chromosome abnormality
- Myelodysplastic syndrome, unclassifiable

- RA
- RARS
- RCMD
- RAEB
- MDS del (5q)
- MDS unclassifiable

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MDS Incidence is age-dependent

MDS Risk Factor: Smoking

Fig. 1. Forest plot of studies on smoking and MDS.
MDS Survival by Sub-Type


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### Benzene and Myelodysplastic Syndrome

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Exposures</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hayes (1997)</td>
<td>7 cases of MDS in exposed (0 unexposed). 5 exposed to &gt;40 ppm-yrs benzene</td>
<td>∞</td>
<td>1.7 - ∞</td>
</tr>
<tr>
<td>Albin (2003)</td>
<td>‘Ever’ vs never exposed</td>
<td>0.95</td>
<td>0.54 – 1.7</td>
</tr>
<tr>
<td>Irons (2010)</td>
<td>&gt;21 ppm</td>
<td>11.1</td>
<td>1.34-92.4</td>
</tr>
<tr>
<td>Schnatter (2012)</td>
<td>&lt; 0.35ppm-yr</td>
<td>1.00</td>
<td>(ref)</td>
</tr>
<tr>
<td></td>
<td>0.35-2.93 ppm-yr</td>
<td>1.73</td>
<td>0.55-5.47</td>
</tr>
<tr>
<td></td>
<td>&gt; 2.93 ppm-yr</td>
<td>4.33</td>
<td>1.41-14.3</td>
</tr>
</tbody>
</table>
Benzene and Myelodysplastic Syndrome

- **Biologic basis for BZ/MDS**
  - MDS, like AML, derives from the myeloid cell line
  - MDS can evolve into AML
  - Both MDS and AML are caused by chemotherapeutic agents
  - MDS is a clonal disease characterized by bone marrow dysfunction, often with associated cytopenias (which are effects of benzene)

- Whether AML is an independent effect or a possible outcome in benzene-induced MDS (or both) remains unresolved.
  - Higher benzene exposures may be necessary for significant risks of AML to occur (e.g. AML may be an independent effect)
  - Since MDS frequently evolves into AML, MDS could be a precursor to benzene-induced AML.
How does benzene exert effects? (mode or mechanism of action)
Irons et al., 2005

23 cases of benzene-induced dysplasia (BID) exposed to high levels of BZ (Shanghai) Most would likely qualify as a type of MDS if not recognized as unique subtype

<table>
<thead>
<tr>
<th>BID vs. de-novo MDS (Myelodysplastic Syndrome)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BID</strong></td>
</tr>
<tr>
<td>26% have normal peripheral blood counts</td>
</tr>
<tr>
<td>No myeloid clonal genetic abnormalities</td>
</tr>
<tr>
<td>Hypocellular bone marrow</td>
</tr>
<tr>
<td>↑ dysplastic eosinophilic precursor cells*</td>
</tr>
<tr>
<td>Severe dyserythropoiesis common*</td>
</tr>
<tr>
<td>Hematophagocytosis*</td>
</tr>
<tr>
<td>Robust T-cell response, clones in half*</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>de-novo MDS</strong></td>
</tr>
<tr>
<td>Peripheral blood abnormalities required</td>
</tr>
<tr>
<td>26% myeloid clonal genetic abn. (e.g. 5q-, 7q-)</td>
</tr>
<tr>
<td>Hypercellular &amp; hypocellular bone marrow</td>
</tr>
<tr>
<td>Less dysplatic eosinophilic precursors</td>
</tr>
<tr>
<td>Dyserythropoiesis less severe/common</td>
</tr>
<tr>
<td>Not usually seen</td>
</tr>
<tr>
<td>More moderate T-cell response, clones rare</td>
</tr>
</tbody>
</table>

* Indicative of an **autoimmune and/or inflammatory** disease process

If true, which candidate genes would indicate autoimmune response?
tumor necrosis factor-α: a candidate gene

• cytokine involved in:
  • immune and inflammatory responses
  • apoptosis checks and balances
  • hematopoiesis (blood formation)

• TNF-α is over-expressed in:
  • rheumatoid arthritis, psoriasis (autoimmune dx’s)
  • aplastic anemia
  • MDS (also some conflicting data)

• located on chromosome 6, four prominent SNPs in promoter region

\[
\begin{align*}
-863 & -857 \\
(C\rightarrow A) & (C\rightarrow T)
\end{align*}
\]

\[
\begin{align*}
-308 & -238 \\
(G\rightarrow A) & (G\rightarrow A)
\end{align*}
\]

TNF-α Transcription Start Site
Study design: Lv et al. 2007, benzene-MDS

95 cases of denovo Myelodysplastic Syndrome (MDS)  
46 cases of Benzene Poisoning (BP)  
23 cases of Benzene-induced Dysplasia (BID)

- **Informed consent**
- **Questionnaire**
- **Blood collected**
- **Genomic DNA**
- **TNF-α SNPs**
- **Examine if TNF-α related to diseases**

Gather workplace BZ data

- % >30 ppm  
- avg. ppm

- **BID:** 100%  
- 305 cases

- **BP:** 65%  
- 123 cases

- Others (MDS): 0%  
- 0 cases

Exclude conditions related to autoimmunity, e.g., arthritis, drug use, nutritional anemia, etc.
results: risks of TNF-α SNPs by disease
Summary of mechanistic studies

- Benzene-induced dysplasia (BID), a type of MDS, involves bone marrow inflammation, abnormal hematopoiesis, and a role for autoimmunity.

- TNF-α has a key role in some autoimmune diseases.

- One of four TNF-α SNPs, namely -238A, is related to certain types of MDS.

- This relationship provides further support for the role of autoimmunity in MDS.
Prospects for useful biomarkers

Susceptibility
• TNF-α 238A genetic polymorphisms

Risk of evolving MDS
• Early autoimmune changes
  • Increase in CD8+ cytotoxic T-cell lymphocytes (CTL)
  • Clonal/oligoclonal expansion of BM CD8+ CTL
    • Can be measured in peripheral blood*
    • Expansion of distinct clones indicate previous response in bone marrow

Potential groups at higher risk

- SNP’s of key genes
  - TNF-α (immune response)
  - MPO (metabolism)
  - NQO1 (detoxification)
- Males > females
  - Slower excretion
- Asian populations
  - Blood effects more prominent – ALDH?
- Smokers
  - Another source of BZ, potentiation?
Summary

• Recent studies show elevated MDS in BZ exposed workers

• MDS a relatively “new” disease, can progress to AML, strong age effect

• Key events for benzene toxicity still unknown, bone marrow inflammation/autoimmunity may be key

• Recent findings could impact OEL’s

• At-risk populations still uncertain

• Cytotoxic t-lymphocytes offer promise as a useful biomarker
Acknowledgements

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Deborah Glass, Monash University Melbourne Australia

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Richard Irons University of Colorado
Malcolm Sim Monash University

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Gong Tang University of Pittsburgh
Min Chen ExxonMobil Biomedical Sciences
Susan Marcella ExxonMobil Biomedical Sciences

Exposure assessment team included:
Dave Verma McMaster University
Tom Armstrong TWA8hr, formerly ExxonMobil Biomedical Sciences
Eileen Pearlman retired, formerly ExxonMobil Biomedical Sciences

• Funders: CONCAWE, API
Resources

• Pooled Analysis study: Journal of the National Cancer Institute
  • http://jnci.oxfordjournals.org/content/early/recent

• More on MDS: American Cancer Society
  • http://www.cancer.org/Cancer/MyelodysplasticSyndrome/DetailedGuide/myelodysplastic-syndromes-what-is-m-d-s

• More on benzene: ATSDR
  • http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=40&tid=14
  • http://www.atsdr.cdc.gov/toxfaqs/tf.asp?id=38&tid=14
Thank-you!
Questions?
back-up/supplemental material
Hematopoiesis and Stem Cell Differentiation
References


Jaffe ES, Harris NL Stein H Vardiman JW (eds.) World health Organization Classification of Tumors: Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues IARC Press: Lyon 2001


Rushton L and Romaniuk (1997) A case control study to investigate the risk of leukaemia associated with exposure to benzene in petroleum marketing and distribution workers in the United Kingdom Occ Env Med 54: 152-166

