Postmortem Alcohol Issues

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Detroit, Michigan
Ethanol is the most frequently assayed and one of the most common drugs detected in a postmortem forensic toxicology laboratory.
## Ethanol by Manner of Death

<table>
<thead>
<tr>
<th>Manner</th>
<th>N Cases</th>
<th>% Positive</th>
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<tbody>
<tr>
<td>Natural</td>
<td>1027</td>
<td>9.5 %</td>
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<tr>
<td>Suicide</td>
<td>139</td>
<td>23.0 %</td>
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<tr>
<td>Homicide</td>
<td>448</td>
<td>30.4 %</td>
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<tr>
<td>Accidents</td>
<td>334</td>
<td>27.5 %</td>
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<tr>
<td>Drivers</td>
<td>90</td>
<td>30.0%</td>
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<tr>
<td>Pedestrians</td>
<td>74</td>
<td>37.8%</td>
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<tr>
<td>Pending Tox</td>
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<td>21.4 %</td>
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</table>

Data - WCMEO, 2002
Analysis is easy
Interpretation is complex
PM Blood?
PM synthesis and diffusion
Analysis

- Conway microdiffusion (non-specific)
  - dichromate + acid + ethanol → chromic

- Enzymatic (ADH)
  - NAD + ethanol ⇌ NADH + acetaldehyde
  - Trapping agent - (hydrazine / semicarbazide)

- Radiative Energy Attenuation (Abbott)
  - NADH + MTT ⇌ NAD + MT-Formazan (diaphorase)
  - MTT = Monotetrazolium dye
Gas Chromatograph - FID

- **Direct Injection**
  - sample diluted with ISTD
  - build up of non-volatile substances
  - increased maintenance, column replacement

- **Headspace Injection**
  - analysis of volatilized alcohols
  - less maintenance
  - can use aqueous calibrators if diluted x10
Specimens

- “Blood” - heart, femoral
- Urine
- Vitreous humor
- Bile
- Liver
- Brain
- CSF
- Gastric
Postmortem Changes

- **Postmortem Loss**
  - evaporation (headspace, temperature)
  - enzyme mediated oxidation
  - Microorganisms

- **Postmortem Synthesis**
  - Decomposition
  - Action of microorganism without decomposition
  - Postmortem absorption of alcohol from GI tract
Postmortem Loss

- **Evaporation**
  - loose specimen lids
  - improper storage (refrigerate or freeze)
  - small sample volume in large container
  - salting out from excess sodium fluoride
    » higher VP, lowers BAC by headspace analysis

- **Oxidation to acetaldehyde via O₂Hb / air**
  - 0.02 mg/dL/day at 4°C; 6 mg/dL/day at 37°C
  - not inhibited by sodium fluoride
Postmortem Loss

- Microbial action
  - microbes capable of using EtOH as a substrate for metabolism
  - aerobic metabolism (minimize headspace)
  - sodium fluoride inhibits most
  - generally avoided at refrigerated temp.

- Fill - Seal - Preserve - Refrigerate
Postmortem Synthesis

- **Species that produce ethanol**
  - 58 bacteria - E. coli
  - 17 yeasts - C. ablicans
  - 24 molds
  - Read Corry, J. Appl Bacteriology 44:1-56, 1978

- **Mechanism of microbial contamination**
  - Exogenous (skin breaks)
  - Endogenous (intestinal bacteria - penetration of intestinal walls to portal vein)
Factors in PM Ethanol Production

- Species of microorganism present
- Substrates available (glucose rich tissues)
  - Liver, Skeletal muscle, Lungs, Heart
  - Liver glycogen $\rightarrow$ glucose postmortem
- Temperature of storage (before/after autopsy)
- Absence of sodium fluoride
PM Production - Findings

- All postmortem cases ~12%
  - Caplan and Levine, 1990

- Decomposed cases ~20%
  - Zumwalt et al., 1982; Gilliland and Bost, 1993
  - Most concentrations <0.07 g%

- Higher #'s have been reported in grossly decomposed, trauma cases

- Blood, no preservative, RT, 4 d, up to 0.15

- Urine, high glucose + yeast, RT 21 d, >1.0
PM Synthesis -v- AM Ingestion?

- Case history - if you're lucky
- Condition of the specimens - notate!
- Types of microorganisms present (?!)
- Atypical distribution of ethanol in multiple samples
- Presence in one specimen, not others
- Presence of other alcohols / volatiles
- Ethanol concentration
Distribution of Alcohol

- Once absorbed – distributed throughout the body
- Distribution in tissues – dependant on the water content of the tissue
# Distribution of Alcohol

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<th>Tissue</th>
<th>Ratio</th>
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<td>Whole Blood</td>
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<td>Plasma</td>
<td>1.18</td>
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<td>Urine</td>
<td>1.28</td>
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<tr>
<td>Bile</td>
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<tr>
<td>Vitreous Humor</td>
<td>1.27</td>
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<tr>
<td>Brain</td>
<td>0.85</td>
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<tr>
<td>Liver</td>
<td>0.56</td>
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</table>
Atypical Distribution

- Often difficult to establish
- Must be postabsorptive
- Vitreous / Blood ratios vary considerably
  - Postabsorptive lags behind
  - Stomach <0.5 g% - postabsorptive (Backer, 1980)
    - mean 1.19 (stomach <0.5%) [0.86-1.72]
    - mean 0.89 (stomach >0.5%) [0.48-2.00]
    - mean 1.17 (postabsorptive with BAC >0.10%); [0.25-1.91], N=205, (Caplan and Levine, 1990).
Atypical Distribution

- Other variables
  - Decomposition - atypical distribution likely
  - Hematocrit (7-64%, Coe & Sherman, 1970)
  - Clotting, collapsed vessels
  - Rigor mortis, body position
    » postmortem blood movement
  - Gastric diffusion
  - Dilution (fluids)
Effect of Dilution (transfusions)

- Falsely lowered ethanol concentrations
  - Applies to blood and other fluids
- BAC can be estimated if volume known
- Based on total body water NOT blood
  - 150 lb. male (Vd = 0.66); 2 L fluid = 4.4 lb.
  - 2 L fluids results in 4.4 lb / 100 lb decrease
  - Dilution = 4.4% (Field, 1993)
- Effect magnified if not circulated
  - Vitreous humor may be useful
<table>
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<tr>
<th>(AM)</th>
<th>(PM)</th>
<th>(VH)</th>
<th>Fluids / Time</th>
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<tbody>
<tr>
<td>0.018</td>
<td>0.073</td>
<td>0.093</td>
<td>5.5 L / 1.75 h</td>
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<tr>
<td>0.120</td>
<td>0.230</td>
<td>0.210</td>
<td>1.7 L / 0.75 h</td>
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<td>0.234</td>
<td>0.071</td>
<td>0.104</td>
<td>1.0 L / 2.5 h</td>
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<tr>
<td>0.141</td>
<td>0.091</td>
<td>NA</td>
<td>2.0 L / 4.25 h</td>
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</tbody>
</table>

(possible fluid infusion near time of death)
Presence in Different Matrices

- Negative urine, vitreous - positive blood
  - probably indicates PM synthesis
  - no vitreous glucose or microorganisms
  - protected from putrefaction and trauma
  - no urine ethanol production except, e.g. glucose + yeast

- Ethanol concentrations usually clinically insignificant
### Paired Specimen Study

- **381 cases with BAC from 0.01-0.04 g%**

<table>
<thead>
<tr>
<th>BAC g%</th>
<th>Positive (VH or U)</th>
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<td>&lt;0.01</td>
<td>54%</td>
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<tr>
<td>0.02</td>
<td>63%</td>
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<td>0.03</td>
<td>73%</td>
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<tr>
<td>0.04</td>
<td>92%</td>
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</table>

- Levine et al., 1993
Postmortem Diffusion

- Requires recent ingestion of alcohol
- Stomach - diffusion and/or regurgitation
  - may affect pleural cavity, pericardial fluids, pulmonary vessels, cardiac blood
- Small intestine (upper)
  - may contaminate surrounding organs
- Trauma - especially stomach or diaphragm
But it’s not a big deal!

- “Most people do not die with a belly full of liquor.” (Sunshine, 1957)
- “Few cases with stomach alcohol concentrations greater than 5g/dL.” (Backer, 1980)
- “Avoid blind sticks” (Logan and Lindholm, 1995).
- Exceptions may exist - use vitreous humor
- Blood from two sources may be useful when the quality of the other sample is called into question
  - Femoral generally collected for drugs anyway
Heart -v- Peripheral Blood

- 68 paired fluoridated specimens; mean
- 74% within 10%; 91% within 20%
- 38% heart blood > peripheral blood
- 31% heart blood = peripheral blood
- 31% heart blood < peripheral blood
- Mean % difference <1.5% for all cases
- 4/6 that were >20% H/P was >1.0
  - all 4 had low P sample volumes incl. 1 absorbing

Isenschrom mid and Hepler, 1998 ToxTalk
Heart -v- Femoral Blood

- Prouty and Anderson, 1987
- 100 paired specimens
- Mean Heart / Femoral ratio 0.98
- 17 cases differed by >20%
  - 6 had H/F ration of >1.0
  - either absorptive or low femoral sample vol.
Putrefactive Products and Exogenous Artifacts

- Methanol, formaldehyde
  - (also form embalming)
- Isopropanol (especially drowning cases)
- Acetone (also starvation, ketoacidosis)
- Others - concentrations low
  - n-propranol (20x < blood), n-butanol, sec-butanol, isoamyl alcohol, isobutanol, acetaldehyde, ethyl ether, phenylethanols
Absorptive Phase

Pulmonary Circulation
- Pulmonary Vein
- Pulmonary Artery

Systemic Circulation
- Left Heart: LA, LV
- Right Heart: RA, RV
- Aorta
- Vena Cavae

Oxygenated
- Up to 40% higher

Deoxygenated
Absorptive State of Drivers

■ Levine & Smialek, 1999
  – Drivers dead within 15 minutes (n=129)
  – 11 U/B < 1.0, absorptive
  – 32 U/B 1.0 -1.2, plateau phase
  – 86 U/B >1.2, postabsorptive

■ WCEMO - absorptive, plateau - V/B often <1.0
  – U and V may be useful in absorptive state
Interpretation Guidelines
O’Neal and Poklis, 1996

- Case History
- Decomposed Cases
  - high EtOH in multiple matrices - probably AM
  - DO NOT extrapolate or interpret behavioral efx
- Multiple Specimens
  - Ethanol should be detected in all
  - Otherwise assume ethanol synthesis
  - (Urine, blood clots - example of exceptions)
Interpretation Guidelines
O’Neal and Poklis, 1996

- Internal Standard - use t-butanol
- Specimen container
  - Preserve with at least 1% sodium fluoride
  - Volume should fill container
  - Tight screw-cap lid
- Blood only cases
  - Consider BAC < or = to 0.03 g% NEGATIVE
2Na_2Cr_2O_7 + 8H_2SO_4 + 3CH_3CH_2OH \rightarrow 2Cr_2(SO_4)_3 + 2Na_2SO_4 + 3CH_3COOH + 11H_2O
### Table of Results

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Sample Name</th>
<th>Methanol</th>
<th>Acetone</th>
<th>Ethanol</th>
<th>Isopropanol</th>
<th>n-Propanol (IS)</th>
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<tbody>
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</tr>
</tbody>
</table>

- **Methanol**: Concentration in G/100 dL
- **Acetone**: Concentration in G/100 dL
- **Ethanol**: Concentration in G/100 dL
- **Isopropanol**: Concentration in G/100 dL
- **n-Propanol (IS)**: Concentration in G/100 dL

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**WCMEO Postmortem Forensic Toxicology Laboratory**
1300 East Warren Avenue
Detroit, MI 48207
### WCMEO Volatiles Report

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Component Name</th>
<th>Time [min]</th>
<th>Rel. RT</th>
<th>Area [μV·s]</th>
<th>Adjusted Amount</th>
<th>ISTD Resp Ratio</th>
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Total: 3868466.32  0.6304  5.5663
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<tr>
<th>Sample Number</th>
<th>Sample Name</th>
<th>Methanol</th>
<th>Acetone</th>
<th>Ethanol</th>
<th>Isopropanol</th>
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