Two Fatalities Involving
Substituted Phenols:
Dinitrophenol and Disophenol

Ray Gary
San Diego County Medical Examiner
2,4-Dinitrophenol (DNP)
What is DNP?

- Yellow crystalline solid; molecular weight = 184.11

- Used commercially in the production of dyes, wood preservatives, photographic developers, explosives and pesticides.

- In the 1930’s DNP was used extensively as a diet pill due to its ability to increase basal metabolic rate.

- Noted side effects include: increased sweating, increased heart rate, weight loss, and a marked increase in body temperature.
How does DNP work?

- Uncoupler of oxidative phosphorylation
- Inhibits the synthesis of ATP in mitochondria
- ATP is burned at a rapid rate
- Body temperature increases dramatically
- Fat is burned as energy
2,4-DNP effects on humans

- Reproductive damage
- Skin / eye irritation
- Nose / throat irritation
- Liver and Kidney damage
- Cardiac disturbances
- Digestive and respiratory tract irritation
- Causes cataracts
- Death
DNP throughout history

- Early 1900’s, European and American scientists found that DNP caused a rise in body temp in lab animals.
- WWI, French munitions workers experienced weight loss from absorption through skin.
- WWII, Hitler used DNP to keep prisoners warm during winter without the need for heating the buildings.
- 1933, Stanford University study declared that 2,4-DNP could cause amazing weight loss.
- 1938, DNP use for the treatment of obesity was banned by the FDA.
DNP popularity in the 1930’s

- Stanford University releases weight loss findings in 1933
- ~100,000 people took DNP during the next 15 months
- Most people lost an average of 1-2.5 lbs/week with no change in diet or exercise
- Many reports of adverse reactions, including cataracts and death
- 1935 epidemic of cataracts began to occur – only in women
### DNP and metabolic rate

<table>
<thead>
<tr>
<th>Dose (mg/kg/day)</th>
<th>Avg. increase Basal Metabolic Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2</td>
<td>13 %</td>
</tr>
<tr>
<td>2.3</td>
<td>20 %</td>
</tr>
<tr>
<td>3.5</td>
<td>31 %</td>
</tr>
<tr>
<td>4.7</td>
<td>50 %</td>
</tr>
<tr>
<td>5.9</td>
<td>56 %</td>
</tr>
</tbody>
</table>

*One individual had 82 % increase in BMR after taking 3.3 mg/kg/day for 182 days.*
DNP and metabolic rate cont.

- Another study found that 3-5 mg/kg/day resulted in raising metabolism 25-30%.
- Could lose 20 lbs of fat in 6 weeks – with NO change in diet or exercise.
## Animal Studies on DNP

<table>
<thead>
<tr>
<th>Species</th>
<th>100% survival dose</th>
<th>100% lethal dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>rats</td>
<td>20 mg/kg</td>
<td>60 mg/kg</td>
</tr>
<tr>
<td>dogs</td>
<td>20 mg/kg</td>
<td>30 mg/kg</td>
</tr>
</tbody>
</table>
2,4-DNP deaths

- 1933 San Francisco newspaper reported the death of a Vietnamese Dr. who overdosed on DNP
  - Body temperature was 110 °F
2,4-DNP deaths cont.

- 80 kg male took 46 mg/kg followed by second dose one week later
  - Temp of 105.7 ºF
  - Pulse of 146 bpm
  - Death occurred 11 hours after ingestion
  - Autopsy showed signs similar to heatstroke
  - Rigor mortis set in 10 min. after death
  - Body temp rose to 115 ºF after death
2,4-DNP deaths cont.

- Woman took 7 mg/kg/day for 5 days
  - Admitted to hospital in comatose condition
  - Temp. of 101.8 °F
  - Pulse of 140 bpm
  - Autopsy revealed:
    - Hemorrhagic lungs, spleen and spinal cord
    - Degeneration of renal tubules and liver cells
    - Segmentation and fragmentation of cardiac muscles
2,4-DNP deaths cont.

- Woman took 2.66 mg/kg/day for 14 days
  - Temp. of 102 °F
  - Pulse of 148 bpm
  - Became comatose and died

- Teenage girl took 1.0 mg/kg/day for 46 days
  - Temp. of 105.6 °F
  - Hospitalization and then death
17 yr. old female (75 kg)
- Ingested 12-15 “diet pills” in suicide attempt
- Temp. at admission was 98.8 °F
- Pulse of 150 bpm
- Temp. rose to 104 °F
- Numerous attempts to lower body temp
  - Cold compresses, ↑ AC, acetaminophen, benzos
- Died ~6 hours after admission
- Postmortem serum DNP = 315 mg/L
Review of DNP deaths

- Deaths occurred in people who ingested 3-46 mg/kg/day for short periods
- Deaths occurred in people who ingested 1-4 mg/kg/day for longer periods
- No deaths reported in studies where subjects were given oral doses ≤ 1.3 mg/kg/day for <14 days
Bodybuilders and 2,4-DNP

- DNP regarded as “Mother of all fat burners”
- “Most effective tool available today for the loss of body fat”
- Can be purchased online ($7/200 mg or $10/300 mg)
- Recommendations when taking DNP include:
  - Sleep with fan pointed towards head at night
  - Wear light colored / loose fitting clothing
  - Wash bedding daily because of profuse sweating
  - Consume lots of water (up to 8 liters per day)
  - Supplements are essential to counter negative effects of DNP
**How does DNP stack up?**

<table>
<thead>
<tr>
<th>Weight loss agent</th>
<th>boost in metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECA Stack (ephedrine, caffeine, aspirin)</td>
<td>3 %</td>
</tr>
<tr>
<td>Clenbuterol / Cytomel Stack</td>
<td>5-10 %</td>
</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>50 %</td>
</tr>
</tbody>
</table>
Circumstances

- 28 year old male college student (6 ft, 212 lbs)
- Found with fever, laying in bathtub full of ice
- Taken to fire dept. then transported to hospital
- Admitted to hospital suffering from confusion, hyperthermia and muscle rigidity
- Subject was conscious upon admission, but died soon after
Circumstances cont.

- Deceased had eaten clam chowder despite shellfish allergies
- Roommate stated deceased was using bodybuilding supplements
- Medication bottles, syringes and yellow powder turned over to Medical Examiner’s Office
- Supplements and steroids were from Mexico
Hospital Admission

- Blood sugar: 123
- Pulse: 140
- Blood pressure: 180/20
- WBCs: 15,800
- Platelets: 54
- Potassium: 11.2
- SGOT: 624, SGPT: 713
- Hospital Tox screen: Negative
- Temperature: 106°F
Autopsy findings

- Jaundiced
- Left ventricular hypertrophy of 1.6 cm
- Multi organ congestion
- Severe pulmonary edema
- Severe cerebral edema
- Hepatic necrosis
- 150 mls blood in stomach, hemorrhagic gastritis
- Evidence of CPR (fractured ribs and sternum)
Toxicology

Routine Tox screening included:

- Alcohols          GC-FID
- Drugs of Abuse    ELISA
- Basic drug screen GC/MS
- Acid/Neutral screen HPLC-DAD
- Urine drug screen Toxi-Lab

*antemortem blood samples used where possible
Toxicology results

- **Alcohols**: Negative
- **Drugs of Abuse**: Negative
- **Basic drug screen**: Diphenhydramine 0.13 mg/L
  - Lidocaine <0.05 mg/L
- **Acid/Neutral screen**: Unknown peak
- **Urine drug screen**: Unknown spot in acid extract
  - Diphenhydramine
- **Yellow powder identified by GC/MS as**: 2,4-Dinitrophenol (DNP)
Area Percent Report

Data File : D:\DATA\RGARY\031204\ID031202.D
Acq On : 12 Mar 2004 10:10
Sample : 04-0470 POWDER
Misc : YELLOW POWDER

Vial : 2
Operator : R. GARY
Inst : 5972 GC/M
Multipl : 1.00
Sample Amount : 0.00

MS Integration Params: autoint1.e
Method : C:\HPCHEM\1\METODS\BASE.M (Chemstation Integrator)
Title :

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ID031202.D BASE.M Wed Mar 17 07:49:00 2004
Library Searched: C:\DATABASE\PMW_TOX2.L
Quality: 96
ID: 2,4-Dinitrophenol

Scan 526 (8.903 min): TS111901.D

@ P773

[Graph showing mass-to-charge ratio (m/z) against abundance with peaks at m/z values such as 53, 63, 79, 91, 107, 154, and 168.]
2,4-Dinitrophenol Extraction

- 500 µl specimen (calibrator, control, or sample)
- 100 µl internal standard solution (phenobarb)
- 500 µl of 1 N HCl and vortex
- 8 mls 1-chlorobutane and mix for 30 min.
- Centrifuge and decant into fresh tubes
- Evaporate at 37°C
- Reconstitute with 200 µl mobile phase (0.031 M Pot. Phosphate buffer / acetonitrile; 85:15)
- Inject onto HPLC column
### HPLC instrument parameters

1. **Instrument:** High Performance Liquid Chromatograph  
   Agilent 1100 series

2. **Instrument Parameters:**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Column</strong></td>
<td>Novapak Phenyl, 2 x 150 mm, 4 μm particle microbore column</td>
</tr>
<tr>
<td><strong>DAD monitoring</strong></td>
<td>200-350, 4nm</td>
</tr>
<tr>
<td><strong>UV wavelength</strong></td>
<td>240 nm</td>
</tr>
<tr>
<td><strong>Mobile Phase Initial Flow</strong></td>
<td>0.6 ml per minute</td>
</tr>
<tr>
<td><strong>Gradient</strong></td>
<td>15% B 0-5 minutes</td>
</tr>
<tr>
<td></td>
<td>28% B 5-10 minutes</td>
</tr>
<tr>
<td><strong>Run Time</strong></td>
<td>10 minutes with 5 minute post time</td>
</tr>
<tr>
<td><strong>Injection Size</strong></td>
<td>30 μL</td>
</tr>
<tr>
<td><strong>Mobile Phase A</strong></td>
<td>0.031 M Phosphate Buffer</td>
</tr>
<tr>
<td><strong>Mobile Phase B</strong></td>
<td>Acetonitrile</td>
</tr>
</tbody>
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2,4-Dinitrophenol by HPLC
Search result of: DAD1, 3.560 (80.5 mAU, Apx)
Library used: C:\HPCHEM\SPECLIBS\BENZO.UVL
Library Name: Benzodiazepines by HPLC-DAD
Parameters: None

Name: dinitrophenol
Match: 990.451
Entry: 64
Time: 3.80
ID#: 0
ID Name: dinitrophenol

*** End of Report ***
# 2,4-DNP concentrations

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Concentration</th>
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</thead>
<tbody>
<tr>
<td>AM blood</td>
<td>28 mg/L</td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>31 mg/L</td>
</tr>
<tr>
<td>Peripheral serum</td>
<td>29 mg/L</td>
</tr>
<tr>
<td>Gastric contents</td>
<td>850 mg</td>
</tr>
<tr>
<td>Urine</td>
<td>53 mg/L</td>
</tr>
<tr>
<td>Vitreous</td>
<td>3.4 mg/L</td>
</tr>
<tr>
<td>Bile</td>
<td>present</td>
</tr>
<tr>
<td>Liver</td>
<td>present</td>
</tr>
</tbody>
</table>
Conclusions

- DNP popularity is growing

- Attractive to individuals who wish to drop weight with NO change in diet or exercise – “magic pill”

- Very dangerous
  - No antidote
  - Narrow therapeutic window
  - Can be bought online without prescription despite FDA regulations
Disophenol
(2,6-diiodo-4-nitrophenol)
What is Disophenol?

- 2,6-diiodo-4-nitrophenol; MW = 391
- Anthelmintic used to treat parasitic infestations in dogs and livestock
- Similar in structure and action to 2,4-DNP
- Uncoupler of oxidative phosphorylation
- Not approved by FDA for human consumption
- No known cases of human fatality involving Disophenol until now
2,4-Dinitrophenol

DNP

2,6-Diiodo-4-Nitrophenol

Disophenol
61 year old Caucasian female

Admitted to hospital with complaints of vomiting, diarrhea, dizziness and joint pain

Decedent left ER but was convinced to return by hospital staff

Nurses noted rapid rise in body temperature

Cooling measures were unsuccessful

Cardiac arrest and death ~9 hours after admission

Decedent exhibited rigor mortis type stiffness within minutes of cardiac arrest
Circumstances cont.

- Self diagnosed Gongylonema infestation
  - Parasitic infestation of oral mucosa
  - Very uncommon in humans
  - Decedent may have been delusional
  - Claims to have identified 3 mating pairs of worms
  - No evidence of infestation
  - Biopsy and postmortem examination both negative

- Ingested Disophenol (2,6-diiodo-4-nitrophenol)
  - Anthelmintic no longer available in U.S.
  - Used to treat hookworms in canines
Disophenol “Prescription”

- After 2 years and multiple M.D.s, decedent found someone to prescribe Disophenol even though it’s not available in the U.S.

- “Rx”: take five 100 mg capsules, repeat dose one week later

- Capsules from Mexico: actually contained 500 mg each

- Decedent ingested five 500 mg capsules (5 X recommended dose)
Autopsy results

- Pulmonary congestion and edema
- Hepatic necrosis
- No gross evidence of parasitic infection
- No microscopic evidence of parasitic infection
- Mild upper gastrointestinal hemorrhage
Toxicology

Routine Tox screening included:

- Alcohols
  - GC-FID
- Drugs of Abuse
  - ELISA
- Basic drug screen
  - GC/MS
- Acid/Neutral screen
  - HPLC-DAD

*Antemortem blood samples used for all screens
Toxicology results

- Alcohols: Negative
- Drugs of Abuse: Negative
- Basic drug screen: Negative
- Acid/Neutral screen: Acetaminophen 7.7 mg/L
  - Unknown peak*

- Blue/white capsule identified by GC/MS as:
  - Disophenol (2,6-diiodo-4-nitrophenol)

*Unknown peak in Acid/Neutral screen later identified as Disophenol
(mainlib) 2,6-Diiodo-4-nitrophenol
Library Searched: C:\DATABASE\NIST98.L
Quality: 94
ID: 2,6-Diodo-4-nitrophenol

Scan 591 (9.147 min): ID011802.D

#105072: 2,6-Diodo-4-nitrophenol
Disophenol extraction

- 0.25 mls sample, calibrator or control into microtubes
- 0.50 mls extraction solvent (acetonitrile + MPPH internal standard)
- Let stand 10 minutes
- Centrifuge at high speed
- Transfer supernatant to autosampler vials
- Inject on HPLC
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Zorbax Eclipse XDB-C18, microbore 2.1 x 150 mm, 5µm particle size column with guard column *</td>
</tr>
<tr>
<td>DAD monitoring</td>
<td>200-350, 4nm</td>
</tr>
<tr>
<td>UV wavelength</td>
<td>230 nm &amp; 208 nm</td>
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<tr>
<td>Mobile Phase Initial Flow</td>
<td>0.45 ml per minute</td>
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<tr>
<td>Gradient</td>
<td>5% B Initial 0.5 minutes 5% B 9 minutes 40% B 18 minutes 45% B 20 minutes 55% B 24 minutes 5% B 27 minutes</td>
</tr>
<tr>
<td>Run Time</td>
<td>30 minutes with 5 minute post time</td>
</tr>
<tr>
<td>Injection Size</td>
<td>12 µL</td>
</tr>
<tr>
<td>Mobile Phase A</td>
<td>15mM Phosphate Buffer</td>
</tr>
<tr>
<td>Mobile Phase B</td>
<td>Acetonitrile</td>
</tr>
</tbody>
</table>
HPLC Calibration Curve

MPPH at exp. RT: 13.808
DAD1 A, Sig=230,8 Ref=350,100
Correlation: 1.00000
Residual Std. Dev.: 0.00000
Formula: \( y = mx + b \)
  \( m: 1.00000 \)
  \( b: 0.00000 \)
  \( x: \) Amount Ratio
  \( y: \) Area Ratio

2,6-Diiodo-4-NP at exp. RT: 15.625
DAD1 A, Sig=230,8 Ref=350,100
Correlation: 0.99997
Residual Std. Dev.: 0.01189
Formula: \( y = mx + b \)
  \( m: 2.17905e-3 \)
  \( b: 1.61925e-2 \)
  \( x: \) Amount Ratio
  \( y: \) Area Ratio
<table>
<thead>
<tr>
<th>#</th>
<th>Meas. Ret.</th>
<th>Compound Name</th>
<th>Area</th>
<th>Amount</th>
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</thead>
<tbody>
<tr>
<td>18.789</td>
<td>MPPH</td>
<td>520.673 0.100</td>
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<tr>
<td>15.616</td>
<td>2,6-Diiodo-4-NP</td>
<td>1609.960</td>
<td>141.158</td>
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</tr>
</tbody>
</table>

$x4 \approx 564 \text{ mg/L}$
Disophenol by HPLC-DAD

Search result of: DAD1, 15.616 (247 mAU, Apx) Ref=15.250 & 16.170
Library used: C:\HPCHEM\SPECLIBS\LCSCRN.UVL
Library Name: LC Screen
Parameters: Entry 200 | -

- **Name**: Disophenol
  - Match: 999.863
  - Entry: 99
  - Time: 15.62
  - ID#: 0
  - ID Name:

- **Name**: 2,6-Diiodo-4-nitrophenol
  - Match: 998.104
  - Entry: 100
  - Time: 15.53
  - ID#: 0
  - ID Name:
Disophenol quantitation

- AM blood (~8 hrs before death) 564 mg/L
- AM blood (~1.5 hrs before death) 296 mg/L
- Peripheral blood 138 mg/L
- Central blood 126 mg/L
- Bile 36 mg/L
- Liver 97 mg/L
- Vitreous 5.3 mg/L
Conclusions

- **Cause of death:** acute disophenol intoxication
- **Manner of death:** accident
- **Significance:** Only known case of disophenol fatality in human