Melatonin Detection in Pediatric Deaths
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What is Melatonin?

Melatonin as a Drug

• Rapidly absorbed
• Half-life: 30-60 min
• Metabolized by CYP1A2
  • 3-12 months: 20-25% of adult levels
  • 1-9 years: 50-55% of adult levels
Pharmacokinetics

Benefits

Benefits

Toxicology

Safety

1. Long-term safety studies are lacking
2. There is extensive evidence from animal and human studies that melatonin acts on multiple physiological systems
   - Reproductive
   - Cardiovascular
   - Immune system
   - Metabolic systems
**Pediatric Exposures**

**Day-care workers used melatonin gummy bears to make 2-year-olds nap, police say**

But staff members at the Day Place, Inc., child-care center faced a simple question: When do you do when you must get a 2-year-old to settle down for a nap?

The employees thought they had an answer, police say. They have been arrested.

They are accused of giving toddlers gummy bears laced with melatonin, an over-the-counter sleep-hormone supplement that induces sleep.

The owner and director of the day-care center called police after finding a nearly empty bottle of melatonin gummy bears, according to a Leslie G.8

**Oregon day-care worker gets 21 years for drugging children so she could go tanning, do CrossFit**

To avoid the risk that the seven infants and toddlers in her care would wake, trudge around and possibly hurt themselves, she said, she drugged them with melatonin.

Netherlin, 39, was sentenced to more than 21 years in prison last week after she pleaded guilty to 11 counts of first-degree criminal mistreatment and one count of third-degree assault in connection with Little Giggles, according to the Oregonian.

**Forensic Method Purpose**

1) To determine if an individual has been exposed to exogenous melatonin without their consent or consent of an authorized parent, guardian, or medical care provider.

2) To evaluate the caretaking environment of an individual.
Sample Preparation

- Sample + ISTD
  - 200 µL blood, s/p, urine, fluid*
  - Melatonin-d4 (ISTD)

- Buffering Step
  - 0.5 mL Sodium Carbonate Buffer (pH 9)

- Liq/Liq Extraction
  - 3 mL MTBE
  - Vortex
  - Centrifuge

- Dry Down
  - 55±5°C

- Reconstitute
  - 200 µL Deionized Water:MeOH (50:50)
  - Vortex
  - Transfer to autosampler vial

*Liquid is matrix dependent

QQQ Method Parameters

<table>
<thead>
<tr>
<th>Column</th>
<th>Pre-Column: BetaBasic 30 Drop-in Guard Cartridge, 2.1 x 10 mm, 3 micron; (75560-012021)</th>
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<td>Synergi Fusion-RP 30 x 2.00 mm, 2.5 micron</td>
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<td>(00B-4423-03)</td>
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<table>
<thead>
<tr>
<th>System</th>
<th>Waters TQD Mass Spectrometer with an ACQUITY Ultra Performance LC</th>
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<tr>
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<tr>
<td>Mobile Phase</td>
<td>A1: H2O 0.1% FA</td>
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<tr>
<td>Flow Rate</td>
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<td>Gradient</td>
<td>Initial</td>
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<tr>
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<tr>
<td>Run Time</td>
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QQQ MRM Parameters

```
237 → 178/163
233 → 174/159
```
Laboratory Results - Forensic Pediatric Cases

Sample Size (N)= 22 Blood Samples

<table>
<thead>
<tr>
<th>AGE</th>
<th>GENDER</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 Months</td>
<td>2 M ; 1 F</td>
</tr>
<tr>
<td>4 Months</td>
<td>1 M</td>
</tr>
<tr>
<td>6 Months</td>
<td>1 M</td>
</tr>
<tr>
<td>8 Months</td>
<td>1 M</td>
</tr>
<tr>
<td>9 Months</td>
<td>2 M ; 1 F</td>
</tr>
<tr>
<td>1 Year</td>
<td>3 M ; 1 F</td>
</tr>
<tr>
<td>2 Years</td>
<td>3 M</td>
</tr>
<tr>
<td>3 Years</td>
<td>1 M</td>
</tr>
<tr>
<td>8 Years</td>
<td>1 F</td>
</tr>
<tr>
<td>9 Years</td>
<td>1 F</td>
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</tbody>
</table>

POLICE (N = 5)

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<tr>
<th>AGE</th>
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<tbody>
<tr>
<td>2 Years</td>
<td>1 F</td>
</tr>
<tr>
<td>5 Years</td>
<td>1 M</td>
</tr>
<tr>
<td>6 Years</td>
<td>1 M</td>
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<tr>
<td>7 Years</td>
<td>1 M</td>
</tr>
<tr>
<td>8 Years*</td>
<td>1 F</td>
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</table>

MEDICAL CENTER (N = 1)

<table>
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<tr>
<th>AGE</th>
<th>GENDER</th>
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<tbody>
<tr>
<td>7 Months</td>
<td>1 F</td>
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None Detected
RL = 1.0 ng/mL
4 positive results
3 positive results
Canceled per agency request

Reported Concentrations

Case 1 - Birth History

- 9-month old female (37-5/7 WGA)
- Birth Weight (7 lbs. 8 oz; 21 inches)
- Mother had longstanding history of drug abuse
- Complications:
  - High risk pregnancy
  - Maternal diabetes mellitus
  - Placenta previa
  - Low birth score
Case 1 - Timeline

- Last known alive: 11/6/13, 9:00PM
- Caretaker ‘partying’: 11/6/13, Evening hours
- Caretaker awoke: 11/7/13, 7:00AM
- Found unresponsive: 11/7/13, 10:00AM
- Pronounced: 11/7/13, 10:12AM

Case 1 - Evidence of Neglect

- Alleged caretaker intoxication
  - “I was so drunk I do not remember my babies last night”
- Allegations
  - Pesticide ingestion – Roach infestation
  - Dextromethorphan ingestion – Drank from sister’s cup, 11/6/13, est. 2:00PM
  - Nicotine ingestion – Consumed ½ cigarette
  - Finding of rubber band in mouth
- Admitted
  - Intentional administration of melatonin
- Unsafe sleeping environment
  - Adult bed
  - Co-sleeping with adolescent sibling (250-300 pounds)
  - Overstuffed bedding
  - Prone sleeping position
- Injuries
  - Evidence of right scalp trauma

Case 1 - CPS

- 4 other children in home; 35 months, 11 years, 13 years, 17 years
- Multiple substantiated maltreatment claims
- 11/18/13 fatality investigation substantiating neglect
Case 1 - Pathology

- Blanched livor, right face and abdomen
- Nasal plume
- Right temporalis hemorrhage with focal subcapsular and subgaleal hemorrhage

Case 1 - Toxicology

- NMS
  - Melatonin blood: 13 ng/mL, cutoff 1.0 ng/mL
  - Melatonin gastric: 1200 mg/mL, cutoff 100 ng/mL

- OCME
  - Negative organophosphates
  - Negative ethanol
  - Negative drugs
  - < 10% COHb
  - +/- wnl vitreous

- COD & MOD: Undetermined
Case 2 - History

- 13-month old male
- Found in warm environment (> 80°F) adjacent to a space heater
- Partial thickness burns to bilateral ventral lower extremities with skin slippage
- Early decomposition with superficial skin slippage
- Petechial hemorrhages over the surface of the thymus and epicardial surface of the heart
- Large amount of white particulate/granular matter in stomach
- Aspiration of gastric content
Case 2 - Toxicology

• NMS Melatonin peripheral blood - 210 ng/mL, cutoff 1.0 ng/mL

Postmortem Panel - negative
Alcohol - negative in blood and vitreous fluid
Electrolytes - consistent with decomposition

COD: Acute melatonin ingestion and complications of thermal injuries to body
MOD: Homicide

Conclusions

• The cases described demonstrate the relevance of melatonin detection in forensic pediatric death investigations
• The presented LC-MS/MS method can successfully be used to evaluate exposure to exogenous melatonin in the pediatric population
• Most instances of accidental overdose in children result in minor clinical effects, and do not require treatment
• The presence of melatonin at a concentration consistent with administration may speak to surreptitious or untoward use
• Future studies: Evaluation of postmortem influences
