Melatonin Detection in Pediatric Deaths

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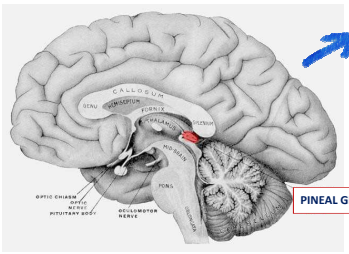
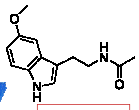
What is Melatonin?





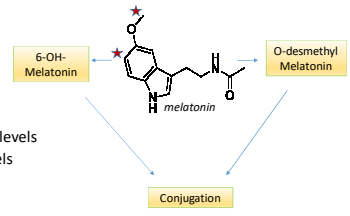
MELATONIN

PINEAL GLAND

http://commons.wikimedia.org/wiki/File:Melatonin.png
https://www.rivearth.com/2014/04/how-to-decalcify-your-pineal-gland-or.html

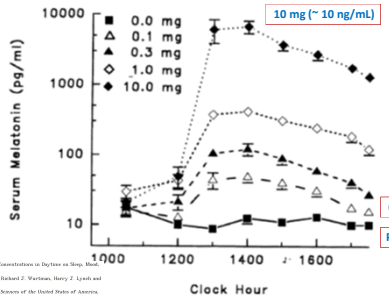
Melatonin as a Drug

- 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



Sonnier M, Cresteil T. Delayed ontogenesis of CYP1A2 in the human liver. Eur J Biochem. 1998 Feb 1;251(3):893-8.
https://commons.wikimedia.org/wiki/File:Melatonin.png

Pharmacokinetics



10 mg (~ 10 ng/mL)

0.1 mg (~ 0.05 ng/mL)

PLACEBO (~ 0.02 ng/mL)

Effect of Inducing Nocturnal Serum Melatonin Concentrations in Daytime on Sleep, Mood, Body Temperature, and Performance
Author(s): Andrew B. Dollins, Irina V. Zhdanova, Richard J. Wurtman, Harry J. Lynch and Mei H. Deng
Source: Proceedings of the National Academy of Sciences of the United States of America, Vol. 91, No. 1 (Dec. 1, 1994), pp. 1824-1828

Benefits

BENEFITS
Inexpensive
Accessible without a prescription
Variety of dosage forms and doses
Lower dose forms are available for children
Considered non-toxic
Promotes sleep: • Chronobiotic

Toxicology

There are very few short-term and no long-term safety data. Toxicological studies have shown that a lethal dose could not be obtained even at extremely high doses.

Researchers gave human volunteers melatonin 6 g each night for 1 month and found no major problems, except for stomach discomfort or residual sleepiness. [1]

Wehr TA. Photoperiodism in humans and other primates: evidence and implications. J Biol Rhythms . 2001;16(4):348-364.

Safety

doi:10.1111/jpc.12840

REVIEW ARTICLE

Potential safety issues in the use of the hormone melatonin in paediatrics

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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

On Parenting

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

But staff members at the Des Plaines, Ill., child-care center faced a simpler question: What do you do when you can't get a 2-year-old to settle down for a nap?

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

They are accused of giving toddlers gummy bears dosed 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 Chicago CBS affiliate WBBM-TV. The owner confronted one of the employees, who allegedly admitted to giving the gummies to children without parents' knowledge.

https://www.washingtonpost.com/news/parenting/wp/2018/03/06/day-care-workers-accused-of-drugging-child-w-with-melatonin-gummy-bears-before-naptime/?noredirect=on&utm_term=.526f9e145d4d

Pediatric Exposures

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 up, trudge around and possibly hurt themselves while she ran errands, Neatherlin drugged them with melatonin.

Neatherlin, 32, 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.

http://www.chicagotribune.com/news/nationworld/ct-oregon-daycare-drugging-20180311-story.html

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)
  - Centrifuge
  - Transfer to autosampler vial

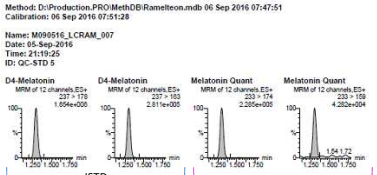
* volume is matrix dependent

Buffering Step → Liq/Liq Extraction → Dry Down → Reconstitute → QQQ/MRM analysis

QQQ Method Parameters

Columns	Pre-Column: BetaBasic 18 Drop-In Guard Cartridge, 2.1 x 10 mm, 3 micron (71503-012101) Synergi Fusion-RP 50 x 2.00 mm, 2.5 micron (00B-4423-B0)		
System	Waters TQD Mass Spectrometer with an ACQUITY Ultra Performance LC		
Column Temperature	50°C		
Mobile Phase	A1: H_2O 0.1% FA B1: MeOH 0.1% FA		
Flow Rate	0.35 mL/min		
Gradient	Time	%A	%B
	Initial	85	15
	1	5	95
Run Time	2.5 min		

QQQ MRM Parameters



ISTD

237 → 178/163 233 → 174/159

Laboratory Results - Forensic Pediatric Cases

Sample Size (N)= 22 Blood Samples

MEDICAL CENTER (N = 1)		
AGE	n	GENDER
7 Months	1	F

None Detected
RL = 1.0 ng/mL

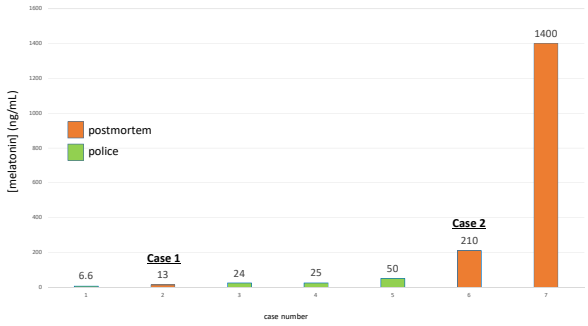
POLICE (N = 5)		
AGE	n	GENDER
2 Years	1	F
5 Years	1	M
6 Years	1	M
7 Years	1	M
8 Years*	1	F

4 positive results
*canceled per agency request

POSTMORTEM (N = 16)		
AGE	n	GENDER
3 Months	2	1 M ; 1 F
4 Months	1	M
6 Months	1	M
8 Months	1	M
9 Months	2	1M; 1 F
1 Year	3	2 M; 1 F
2 Years	3	3 M
3 Years	1	M
8 Years	1	F
9 Years	1	F

3 positive results

Reported Concentrations



Case 1 - Birth History

OFFICE OF THE CHIEF MEDICAL EXAMINER

- 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 praevia
  - 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 subscapular 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

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  < 10% COHb
  +/- wnl vitreous

COD & MOD: Undetermined

Case 2 - History

Central Indiana Forensic Associates, LLC

- 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 - Scene

Case 2 - Scene

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

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- 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 decompositional change

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[1]
- The presence of melatonin at a concentration consistent with administration may speak to surreptitious or untoward use
- Future studies: Evaluation of postmortem influences

Forrester MB. Melatonin exposures reported to Texas poison centers in 1998-2003. Vet Hum Toxicol. 2004 Dec;46(6):345-6.

QUESTIONS?

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https://www.huffingtonpost.com/2014/04/26/counting-sheep-fail-asleep_n_5169807.html