



The Detection of Methadone in Fly Larvae

Heidi Sanderson BSc
(Heidi.5.Sanderson@gsk.com)

Presented by Jennifer Button BSc

IATDMCT Nice 2007

Case Study

- 🦟 In May 2004 a 44 year old woman was found dead in bed (Believed to have been dead at least 2 weeks)
- 🦟 Flat was secure; non-suspicious
- 🦟 History of asthma and IV drug abuse
- 🦟 Ventolin inhalers and empty methadone bottles
- 🦟 Liver and fly larvae were submitted to the laboratory for toxicology screening

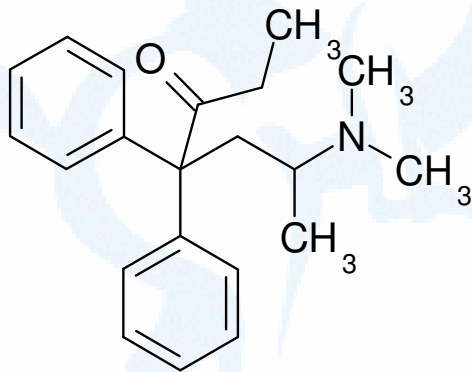
Results

	Liver (mg/kg)	Fly Larvae (mg/kg)
Methadone	5.5	None detected
EDDP	Positive	Positive
Dihydrocodeine	0.2	0.1
Diazepam	3.1	0.6
Desmethyldiazepam	4.4	0.6
Oxazepam	0.27	0.3
Temazepam	0.5	None detected

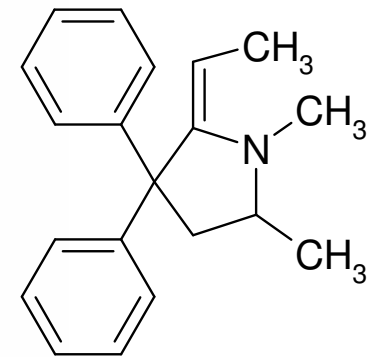
Methadone and EDDP

- Synthetic opiate
- Metabolises to EDDP and EMDP
- Distribution in body:

Larger conc. in liver, kidneys & lungs than in blood



Methadone



EDDP

Questions...

- Can methadone be detected in fly larvae?
- Can fly larvae metabolise methadone to EDDP?

- If so, how rapidly can fly larvae metabolise methadone into EDDP?
- How long can methadone be detected in fly larvae after removal from food source?

Spiking of Liver and "Fly Catching!"

- Liver spiked with methadone
- Ox liver, chopped and soaked in saline phosphate buffer pH7
- Mixed for three days at room temperature - mean concentration 4mg/kg
- Untreated liver was used to 'catch flies'

Distribution, Rearing and Sampling

- Three batches of fly larvae:
 - Solely methadone spiked liver
 - Initially methadone spiked liver; blank liver from day six
 - Solely blank liver - Control
- Approximately 50 eggs per plate
- Maintained at room temperature
- Sampled each day until day 12
- Some allowed to pupate



Preparation of Fly Larvae

- Sacrificed by immersion in boiling water for approximately 10 seconds
- Copiously washed with deionised water, then dried
- Ground down single fly larva with 10 fold volume of deionised water using a pestle and mortar
- Stored at approximately 4°C for a maximum of 24 hours prior to analysis

Extraction of Fly Larvae Homogenate

Internal standard, 1M sodium hydroxide (0.5mL) and MTBE (4mL) were added to the larval homogenate (0.5mL)

The analytes were back extracted into of 0.1M phosphoric acid (0.25mL)

The samples were re-extracted into MTBE (0.2mL) after the addition of 1M sodium hydroxide (0.1mL)

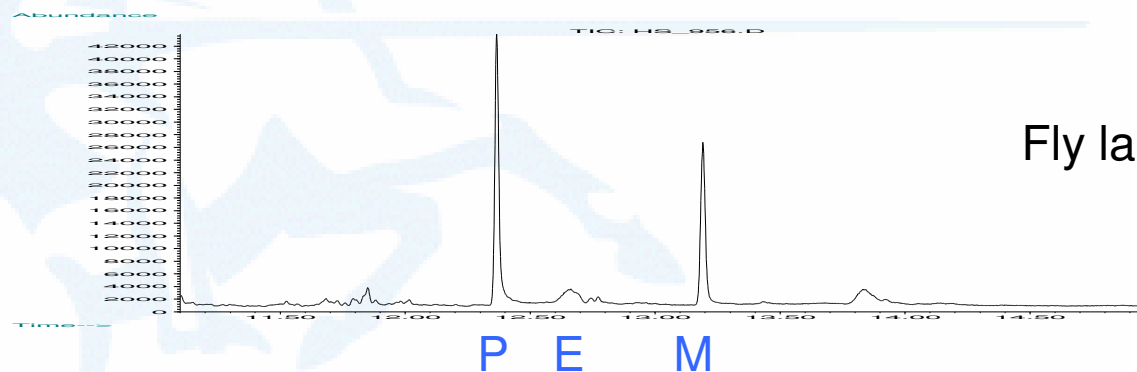
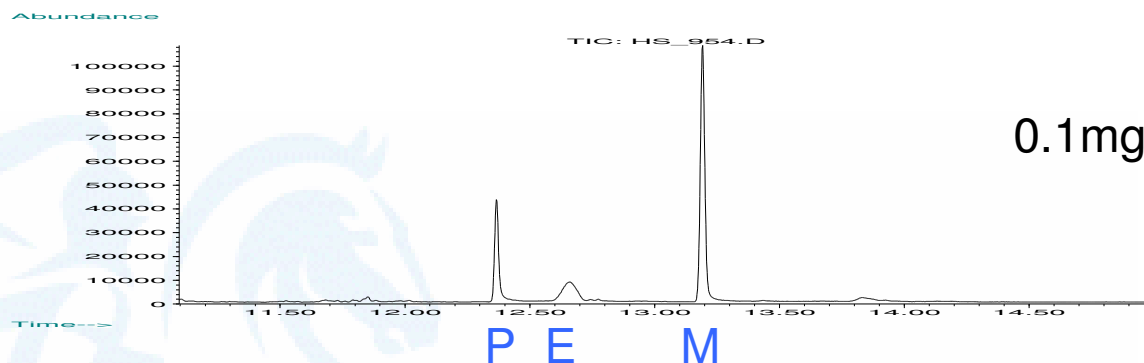
Analysed using GC-MS

Analytical Details

GC-MS

- System: HP 5890 GC interfaced to an HP 5973 MS
- Column: HP-5MS (30m x 0.25mm i.d 0.5 μ m film thickness)
- Ionisation: Electron impact ionisation
- Mass analyser: Single quadrupole
- Carrier gas: Helium
- Injector temperature: 250°C
- Detector temperature: 280°C
- Initial column temperature: 70°C for 6mins, ramped at 40°C/min to 280°C and held for 2mins

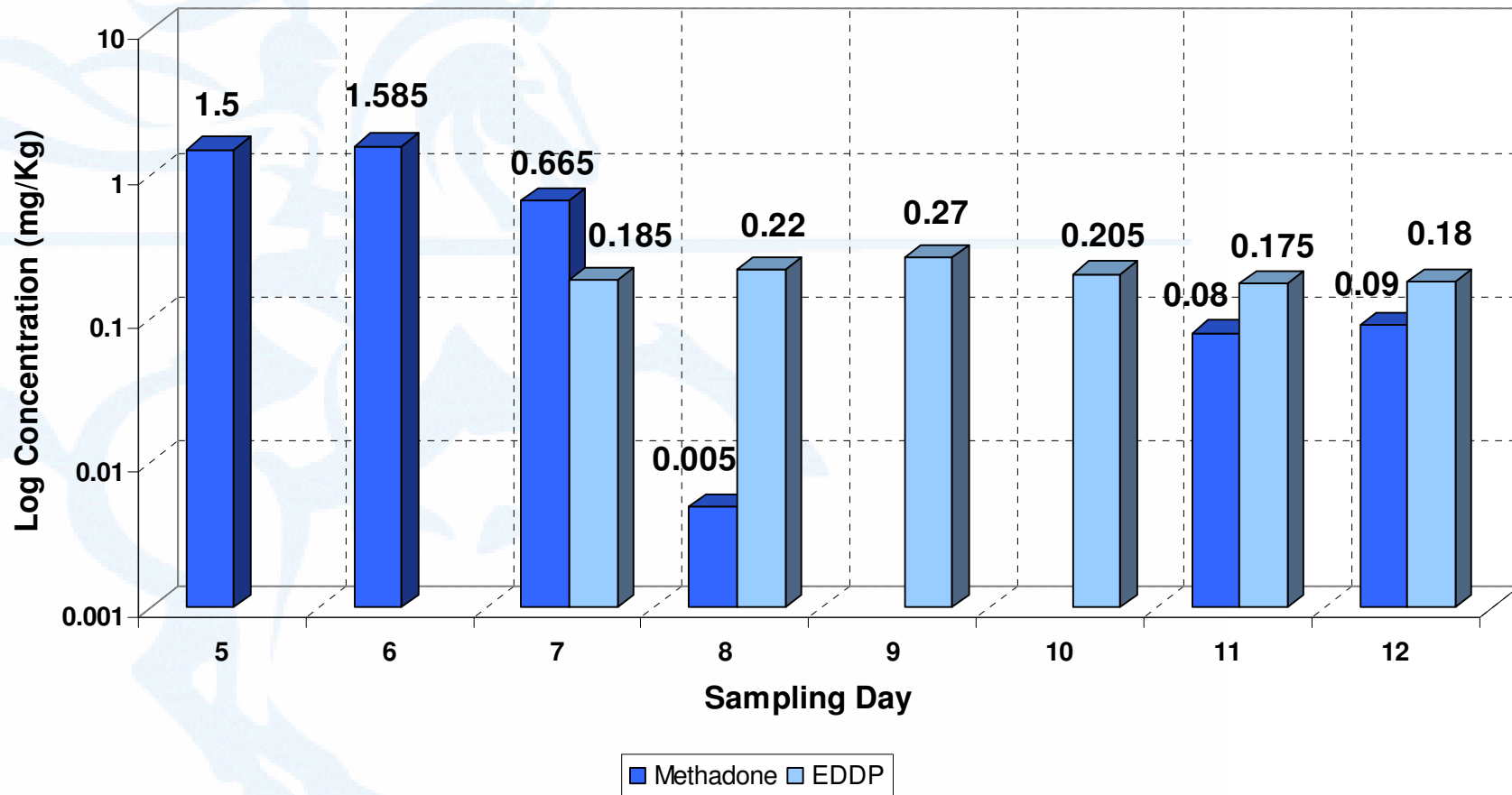
Chromatograms



	R.T	Principle ion
Methadone	13.20	72
EDDP	12.68	277
Pyribenzamine	12.37	91

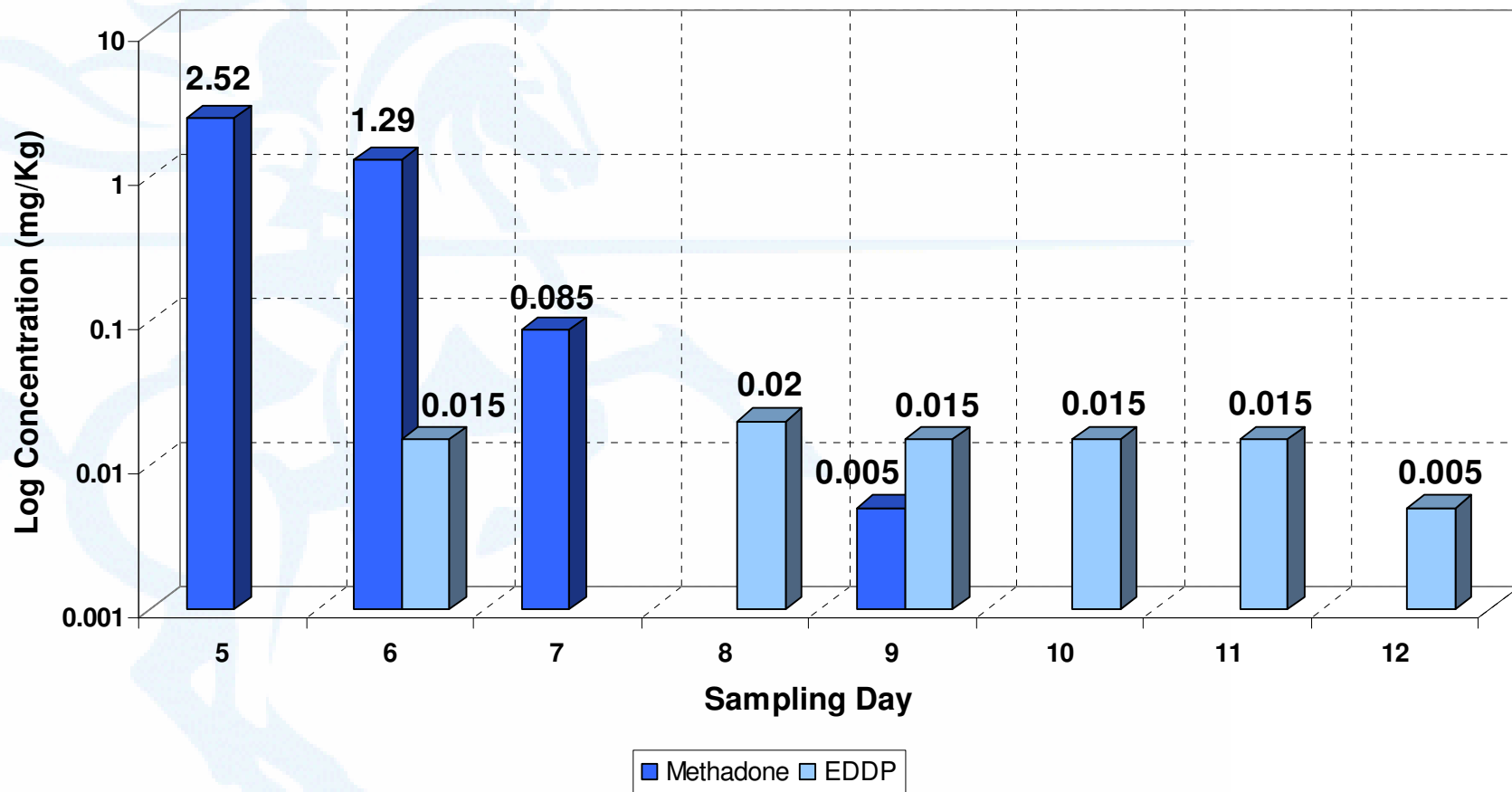
Results

Average Concentrations of Methadone and EDDP From Both Samplings of Fly Larvae Reared Solely on Methadone Spiked Liver



Results

Average Concentration of Methadone and EDDP from both Samplings of Fly Larvae Reared on Methadone Spiked Liver Followed by Blank Liver from Day Six



Adult Flies

Identified by microscopy as *Calliphora Vicina*

Observations:



	Methadone spiked liver	Blank liver
Average length	7.93mm	8.36mm
Colour	Pale blue	Dark blue
Abdominal shape	Rounded	Pointed

Conclusions

- Methadone can be detected in fly larvae.
- Methadone can be detected for up to 3 days after removal from a spiked food source.
- The appearance of detectable concentrations of EDDP occurs at around days 6 to 7.
- Fly larvae do not appear to bioaccumulate methadone or EDDP.
- Methadone appears to affect the morphology of flies.

Initial Results

	Liver (mg/kg)	Fly Larvae (mg/kg)
Methadone	5.5	None detected
EDDP	Positive	Positive
Dihydrocodeine	0.2	0.1
Diazepam	3.1	0.6
Desmethyldiazepam	4.4	0.6
Oxazepam	0.27	0.3
Temazepam	0.5	None detected

Conclusions

Advantages of larval analysis

- Larvae sampling is easy
- Offers an alternative in the absence of routine samples
- Preparation and extraction is the same as with human tissue.
- Toxicological analysis of living material is always preferential.
- No emulsions formed as is sometimes the case with human tissue.
- Fewer endogenous peaks.

Fly larvae are unreliable for quantitative toxicological analysis.

Acknowledgements

- **Forensic Toxicology Service:**
 - Jennifer Button
 - Denise McKeown
 - Terry Lee
 - David Holt
- **Westminster Public Mortuary**
 - Terry Mugridge
- **London South Bank University**
 - Alan Beeby

Questions?

