THE TOXICOLOGICAL INVESTIGATION OF FENTANYL AND OTHER OPIOID RELATED DEATHS

Graham R. Jones, Ph.D., F-ABFT
Chief Toxicologist
Office of the Chief Medical Examiner
Edmonton, Alberta, Canada

A stack of cash seized by police as part of an investigation into a large drug-trafficking ring in Edmonton was displayed by the Edmonton Police Service on July 28, 2017. *SUPPLIED / EDMONTON POLICE SERVICE*

Edmonton police make largest fentanyl pill seizure in Canadian history

The Edmonton police have seized 130,000 fentanyl pills in what is being hailed as the largest fentanyl pill seizure in Canada.

Items seized…

- — 4 kg cocaine: street value $129,000
- — 8 kg methamphetamine: street value $52,000
- — 834 grams cannabis extract: street value $58,000
- — 130,000 fentanyl pills: estimated $3.9 million
- — 113 grams carfentanil: street value $14,000 (>5M fatal doses)
- — 658 grams fentanyl-laced powders: street value $115,000
- — 100 kilograms of buffing agent
- — More than $1 million in Canadian currency
- — *Four portable cement mixers, 100-litre models*
- — Two pill presses (each 5000 pills per hour)

Fentanyl – What does it do?

- Fentanyl is an '**opioid**' ~100 x more potent than morphine
  - By definition, an opioid is an **agonist** molecule that interacts with the **mu-opioid receptors** (primarily in the spinal cord and brain)
- All opioids cause (with varying degrees of potency):
  - Analgesia
  - Sedation
  - Anesthesia
  - After sufficient dose sedation can progress to **respiratory depression**, loss of consciousness, coma and death

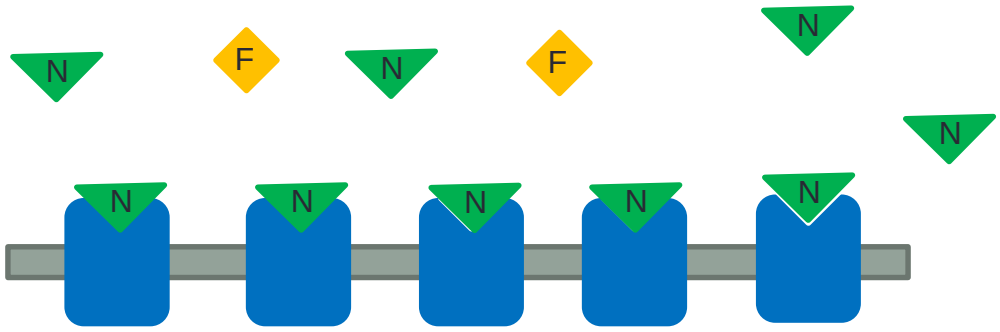
Death is usually the result of depressed (or cessation of) respiration

Medical uses of fentanyl

- Short term anesthesia
  - Acute surgical procedures such as intubation (i.e. inserting an airway)
  - 50 – 200 ug intravenous
- Induction of anesthesia
  - Often with midazolam
- Adjunct to anesthesia in longer surgical procedures
  - Dose depends on type of surgery
  - Airway must be supported for higher doses and longer surgeries
- Transdermal patches for long-term chronic pain
  - 12.5, 25, 50 and 100 ug/hour
  - NOT for acute or post-surgery pain
- Sublingual lozenge for breakthrough pain
  - Dose 0.1 – 1.6 mg

Reversing agents: Naloxone

- Most common is **naloxone** (trade name **Narcan**)
  - Competitive **antagonist** binds the **mu-opioid receptor**, displacing opioids that are already bound there, **without activating** the receptors (i.e., **no analgesia**)



No activation

Reversing agents: Naloxone

Naloxone **half-life ($t_{1/2}$)** ranges from **30 to 80 minutes**

- **Fentanyl $t_{1/2}$ = 3-12 hours**, so repeat injection often required
- If minimal or no response within 2–3 minutes, dosing may be repeated every 2 mins to a maximum 10 mg
  - (e.g. 0.4 mg per injection → 25 injections for 10mg)

Minimal toxicity if opioids not present

- (i.e. low risk administration)

Administration intravenous or intramuscular

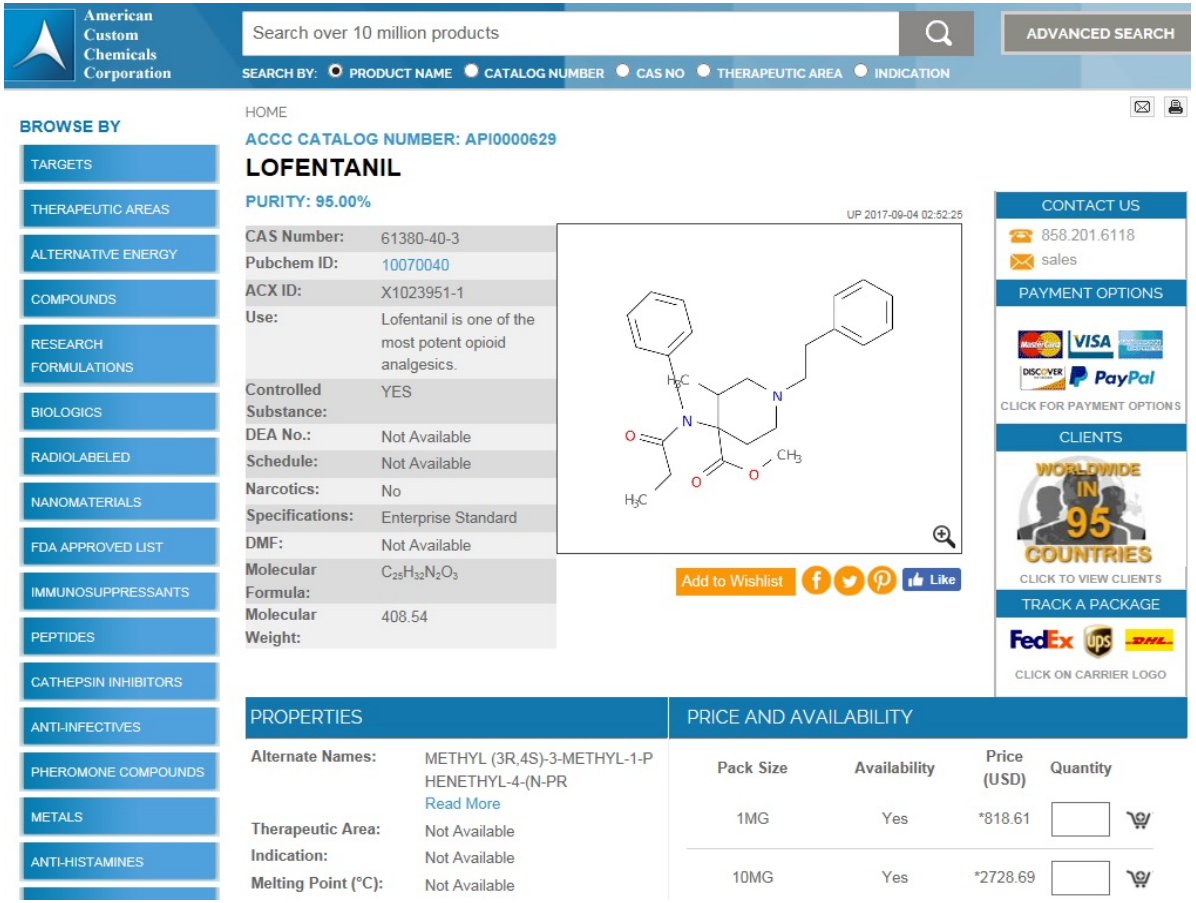
- Now intra-nasal formulation
- Orally – very poor oral bioavailability, takes too long to act

Naloxone use is…

- Useless if it is not administered
  - Difficult if the person is using opioids alone
  - A partner/friend should not assume that the user can just “sleep it off”
  - Many deaths where naloxone is available but not administered
- Marginally useful if a person is abusing opioids alone
  - Why?

LOFENTANIL: A problem if it goes mainstream

- Slightly more potent than carfentanil and a long half life
- Likely to be more difficult to ‘reverse’ with naloxone



Opioid Tolerance

Tolerance

- The ability to withstand increasing doses with static or decreasing side effects

- Tolerance can enable a person to take doses of an opioid that would kill most non-tolerant persons
  - Tolerance is “relative” (i.e. dose related), **not absolute**

- Applies to any opioid

- Cross-tolerance from one opioid to another occurs
  - E.g. if a person develops tolerance to heroin, they will also have a degree of tolerance to fentanyl
  - The degree of cross-tolerance can be difficult to predict

‘Medical’ Tolerance of Fentanyl

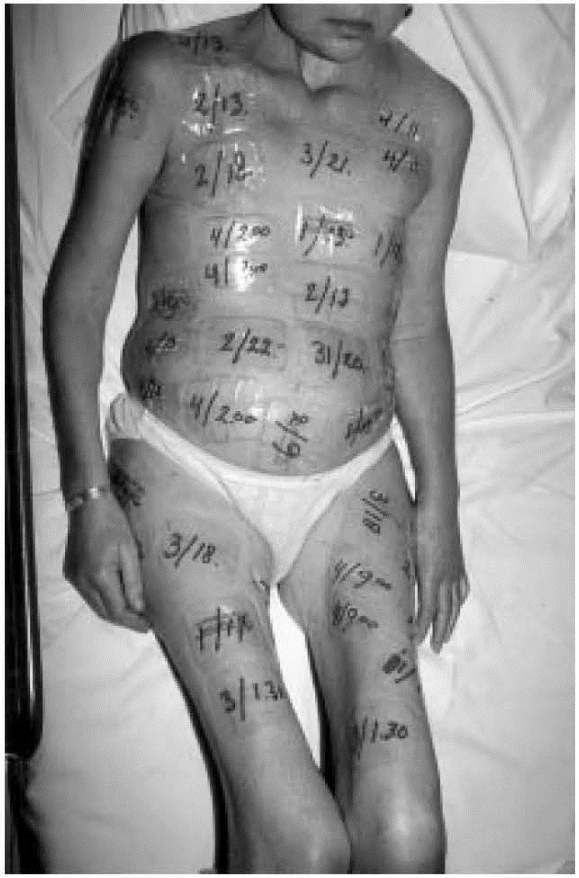


FIG. 3. Patient, after admittance, with a total of 34 fentanyl TTS patches of 100 μg/h located on the anterior and posterior body surface.

- Starting dose 25 ug/hour for chronic pain
- Potentially fatal for non-tolerant patients – 100 ug/hour
- This patient had **34** x 100 ug/h fentanyl patches
- No clinically significant respiratory depression!

European Journal of Pain (2001) 5: 325±331
Inefficacy of high-dose transdermal fentanyl in a patient with neuropathic pain
Bleeker, Bremer, Dongelmans, van Dongen and Crul

Addiction Potential: High

- Reputedly much higher addiction potential than for heroin
- Faster acting, fast euphoria, shorter duration of action
- Readily available to many hospital-based physicians and nursing personnel
  - Opioid of choice for addicted medical professionals

How is fentanyl abused?

Snorting: powder or crushed tablet

'Smoking'

TD patch: inject, chew

Injecting

Less-conventional modes of abuse

Nasal Spray

E-Cigarette

Blotter carfentanil being sold on Winnipeg streets as 'drop dead,' police say

Police chief says he's concerned by market in Winnipeg for deadly opioid

By Bartley Kives, CBC News Posted: Jun 09, 2017 11:29 AM CT | Last Updated: Jun 09, 2017 12:52 PM CT

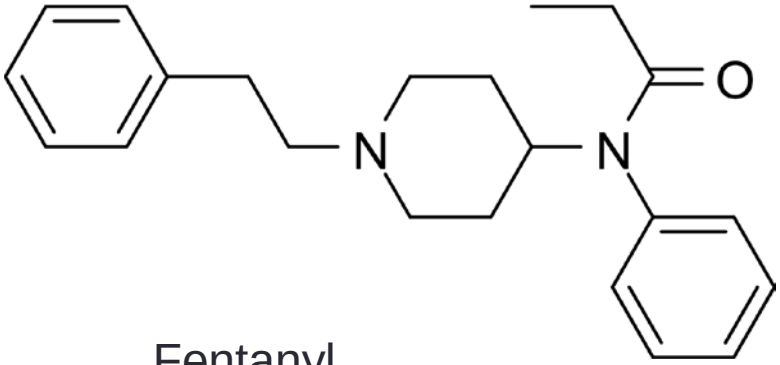
Blotter carfentanil seized by police in September. (Winnipeg Police Service)

Is fentanyl “new”? - No!

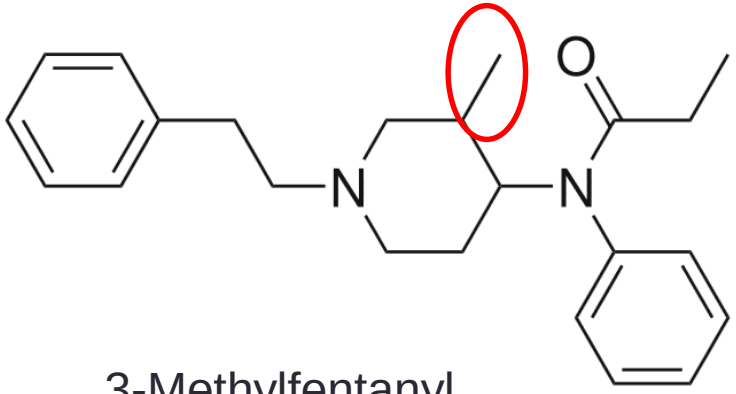
- Developed in 1960 by Dr. Paul Janssen
- Marketed and increasingly used in the 1960s by Janssen Pharmaceutica (trade name Sublimaze)
- Properties of fentanyl and other analogues studied repeatedly with animal testing in 1970-80s
  - Analogues included **carfentanil and 3-methylfentanyl**
- Fentanyl and some of the original analogues are still in legitimate use
  - sufentanil, alfentanil, remifentanil; **carfentanil** (not humans)
- Illicit fentanyl outbreaks cause many deaths in late1970s and 80s

What is an “analogue”?

- “analogue” means a substance that, in relation to a controlled substance, has a substantially similar chemical structure
  - e.g. Schedule I section 16: Fentanyls their salts, derivatives and analogues and salts of derivatives and analogues



Fentanyl



3-Methylfentanyl

Fentanyl analogues today?

- ***At least*** 70 fentanyl analogues “known”
- Many (most?) available for purchase through the Internet as ‘**research chemicals**’
- Many more (hundreds) could theoretically be synthesized
- >30 Different fentanyl analogues screened for as part of the Alberta OCME ‘fentanyl assay’
  - acetylfentanyl, butyrylfentanyl, **carfentanil**, furanylfentanyl, cyclopropylfentanyl, fluoroisobutyrylfentanyl, methoxyacetylfentanyl and 3-methyfentanyl found in Alberta OCME casework

NPS Multi-drug death

- Man in his 40s with a history of drug use was found dead. Significant cardiac and liver disease at autopsy.
  - Postmortem blood
    - carfentanil = detected
    - cyclopropyl-F = detected
    - methoxyacetyl-F = detected
    - fluoroisobutyryl-F = detected
    - U-49900 = detected
    - MDMA = detected
    - methamphetamine = detected
    - alprazolam = detected
- Testing in progress

Fentanyl delayed death

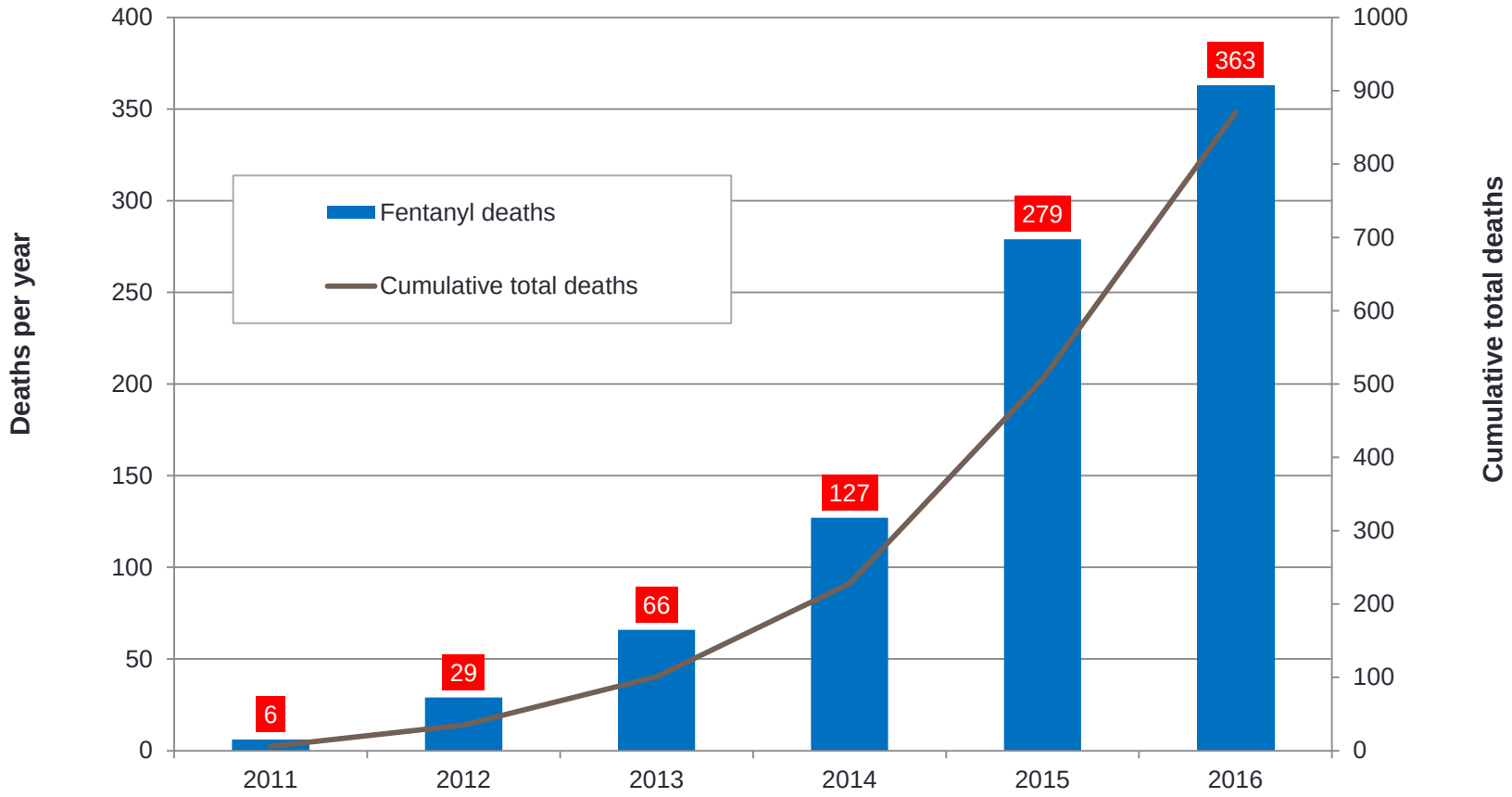
- Man in his 20s with a history of drug use was found unreasonsive by GF and taken to hospital. Life-support withdrawn after a few hours. Delayed hypoxic brain death!
  - Admission blood
    - fentanyl = 4.6 ug/l
    - xylazine = 3.5 ug/l
  - Admission plasma (above hep-gel)
    - fentanyl = 1.2 ug/l
    - xylazine = <LOD
- Initial LC/TOF screen on admission plasma (6 days post death)

Fentanyl 2016/17

- Used to see mostly after medical intervention and patch-related fentanyl deaths (e.g. through 2011/12)
  - Patches stolen, otherwise diverted and abused

- Now mostly illicit non-patch related
  - Used to be mostly fake green ‘oxycontin’ tablets crushed or ‘shaved’ and snorted; some injected’ tablets
  - Now mostly powder cut with caffeine and some with heroin and/or carfentanil

Apparent fentanyl* drug overdose deaths 2011-2016

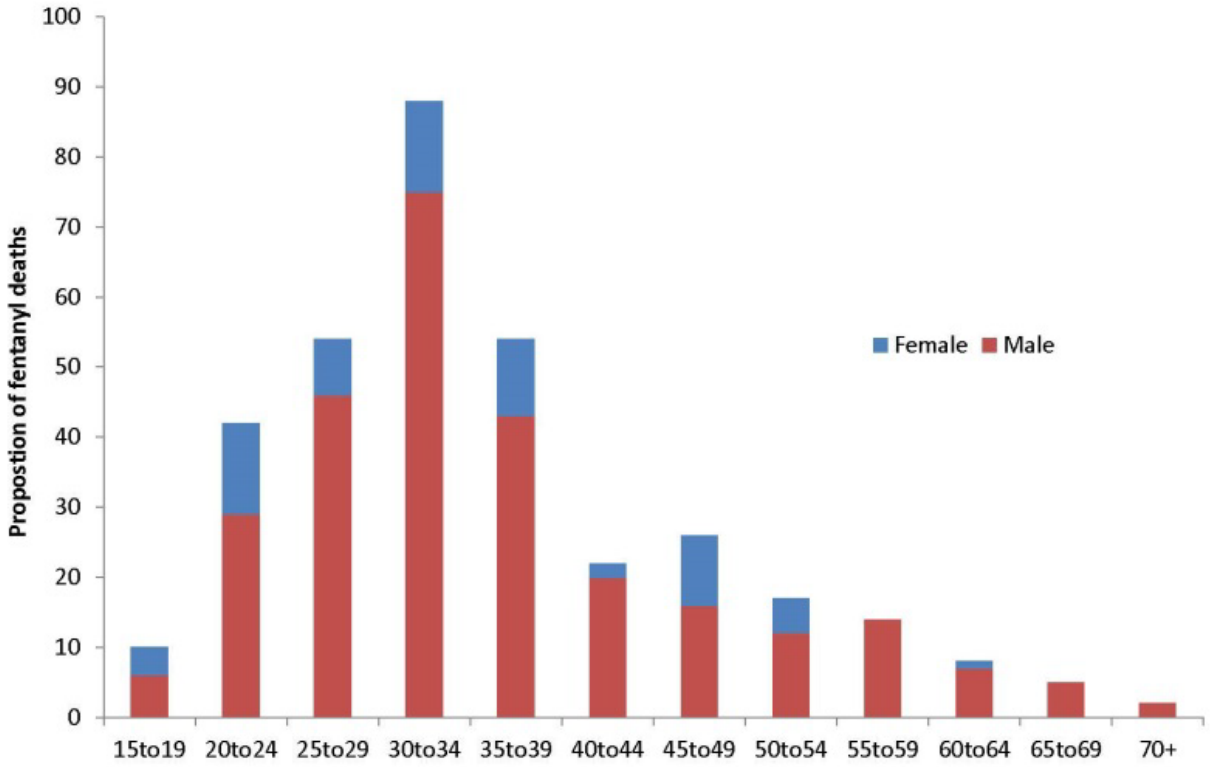


*Includes carfentanil and other analogues

Were are the deaths occurring?

- The majority of deaths are occurring in major urban centers .
  - **91 %** of the deaths in 2017 so far occurred in Calgary, Edmonton, Red Deer, Grande Prairie, Fort McMurray, Medicine Hat, and Lethbridge.
  - **75%** cent occurred in the largest cities, Calgary (**43%)** and Edmonton (**32 %**).
- While these deaths are occurring throughout the cities, the “hot zone” are the centralized urban areas.
- **BUT fentanyl/opioid abuse is a problem across all of Alberta!**

Deaths due to an apparent drug overdose related to fentanyl, by sex and age. Jan 1, 2016 - Dec 31, 2016*



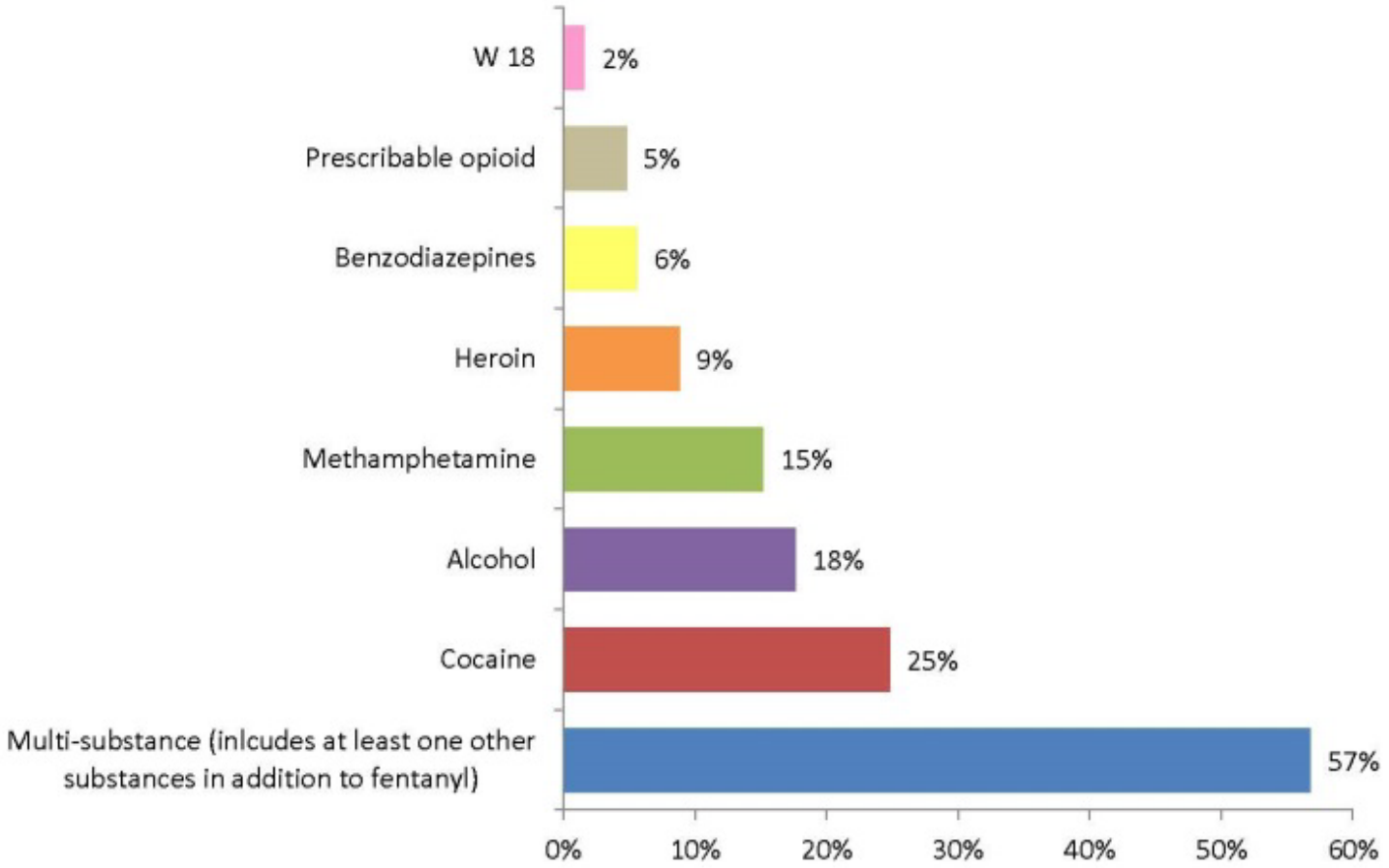
- 80 per cent of deaths due to an apparent drug overdose related to fentanyl were among males. The majority (48 per cent) of these deaths occurred among males spanning the ages of 25 to 39

*Opioids and Substances of Misuse Alberta Report January 30, 2017

Fentanyl alone? – Rarely.

- Almost all fentanyl cases include a stimulant
  - Usually cocaine, some have methamphetamine, a few have both
- Many fentanyl related deaths include ethanol and prescription drugs
- ***Combining opioids with alcohol, benzodiazepines or other depressant prescription drugs is very dangerous***
- About 35% 2015 cases include xylazine
  - Veterinary sedative
- Also:
  - Illicit fentanyl and other opioid analogues, including carfentanil and U-series opioids (e.g. U-47700)
  - Increasingly in combination with heroin

Proportion of deaths due to an apparent drug overdose related to fentanyl, by additional substances contributing to cause of death Jan 1, 2016 – Dec 31, 2016*



*Opioids and Substances of Misuse Alberta Report January 30, 2017

Some perspective on fentanyl dose…

- **Estimated fatal dose of fentanyl**
  - **0.5 – 1.0 mg** (presuming low tolerance and IV administration)
    - will be **lower** with alcohol or depressant drugs
    - will be **higher with tolerance, >2-3 mg**

- 1 mg is not a lot of powder!

  - Regular strength Tylenol contains 350 mg acetaminophen
  - ‘Oxy80’ contains (accurately) 80 mg of oxycodone

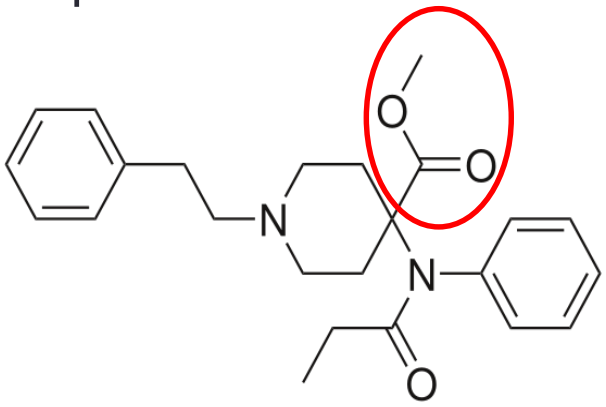
  - ‘**fake Oxys**’ contain **variable amounts of fentanyl** (1-4 mg)
  - But not always fentanyl. May be carfentanil or other drug

CARFENTANIL

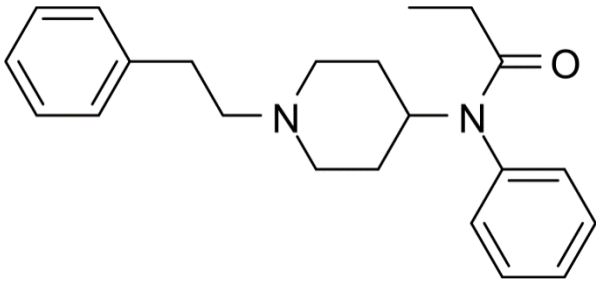
The drug we never thought anyone would be stupid enough to traffic!!

Carfentanil

- Estimated 100x more potent that fentanyl (based on animal studies)
- Very restricted veterinary use for large animals (not used now)
- Implicated in Chechen separatist hostage deaths



Carfentanil



Fentanyl

When did carfentanil become an abused drug?

- Reports of carfentanil abuse in Latvia 2012 & 2013, also Vilnius, Lithuania in 2013
- May 2016: 4th International Conference Novel Psychoactive Substances discussed carfentanil abuse in Europe.
- June 27 2016: 1 kilo of carfentanil seized by Canadian Border Services in Vancouver, destined for Calgary (~50 x 10_6 doses)
- July 2016: start(?) of media reports of deaths from carfentanil tainted heroin in Ohio, later Michigan, Florida
- August 2016: first known Alberta death due to carfentanil

Dose of carfentanil??

Carfentanil potency?

- Widely published: 100x fentanyl and 10,000x morphine
  - Almost impossible to verify in humans – no studies
  - Data relies on small animal studies
- 1976 carfentanil i/v in Wistar rats
  - 10,031x relative to morphine (but fentanyl 292x relative to morphine)
  - Van Bever WF, Niemegeers CJ, Schellekens KH, Janssen PA. N-4-Substituted 1-(2-arylethyl)-4-piperidinyl-N-phenylpropanamides, a novel series of extremely potent analgesics with unusually high safety margin. Arzneimittelforschung. 1976;26(8):1548-51.
- 1988 carfentanil epidural in rats
  - 12x to 14x relative to fentanyl
  - Meert TF, Lu HR, van Craenndonck H, Janssen PA. Comparison between epidural fentanyl, sufentanil, carfentanil, lofentanil and alfentanil in the rat: analgesia and other in vivo effects. Eur J Anaesthesiol. 1988 Sep;5(5):313-21.
- One review estimates carfentanil is 20 – 30x more potent than fentanyl
  - Mather LE. Clinical pharmacokinetics of fentanyl and its newer derivatives. Clin.Pharm. 1983; 8: 422-446
- Immobilizing pachyderms and large ungulates: carfentanil 1 – 10 mg
  - V. de Vos. Immobilization of free-ranging wild animals using a new drug. Veterinary Record 1978; 103: 64-68.
- Immobilizing cervidae (moose, elk, deer): carfentanil 0.005 – 0.02 mg/kg (350 – 1400 ug/70 kg) – BUT reversing agent required!!
  - http://wildpharm.com/ carfentanil data sheet
- 'Bottom line' – carfentanil is a highly potent opioid!

Detection in the toxicology lab?

- After talking with colleagues and available information, we ordered carfentanil in late July 2016
- Detected carfentanil in drug paraphernalia in two unrelated cases on September 26th
- Received the first carfentanil-d0 and -d5 standards October 3rd
- First carfentanil assay online October 5th
- But how do we “screen” for carfentanil if drug paraphernalia is not available?

Difficulty of screening for carfentanil in blood

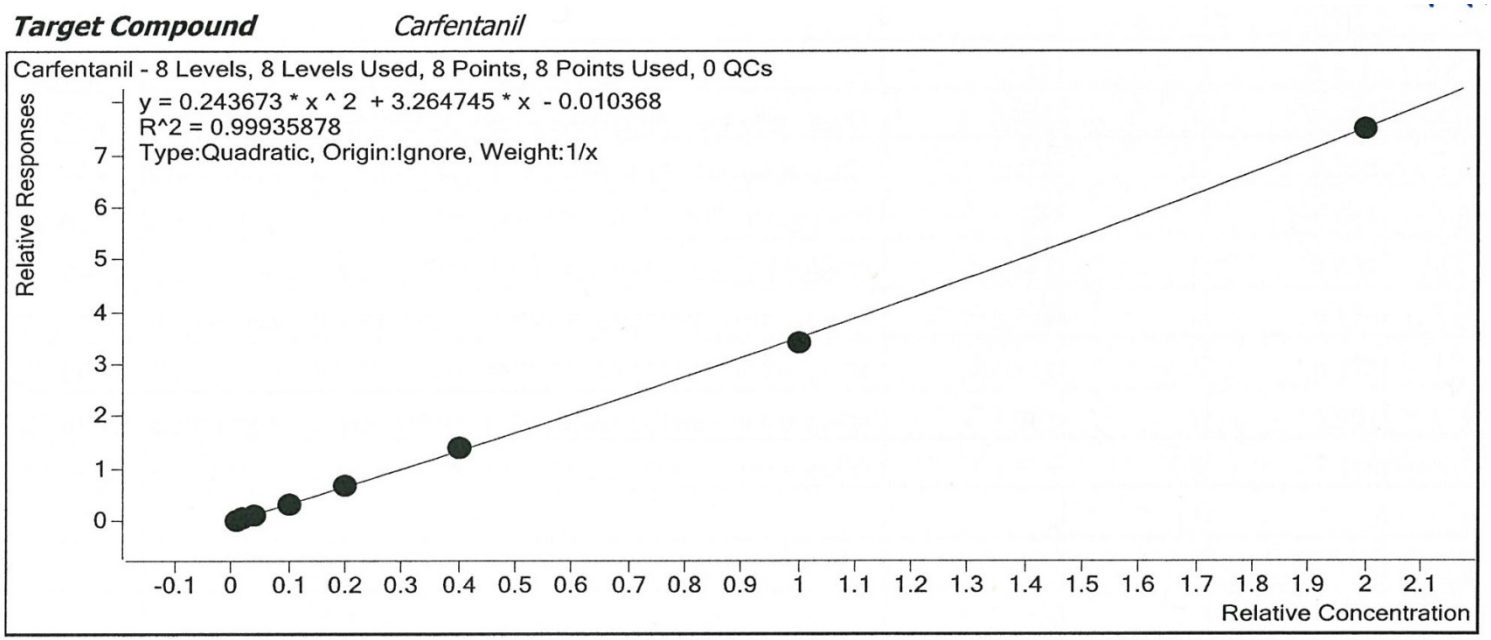
- Concentrations of carfentanil in blood were expected to be in the range 0.01 – 0.10 ng/mL
- Too low for GC/MS or most LC/TOF screens
- ELISA assay was available, but not sensitive enough
- Could detect by LC-MS/MS on one instrument, but difficult to screen a large number of cases efficiently
- Resolution: reinject all LC/TOF screen extracts on the LC-MS/MS (6460)

Carfentanil quantitation

- 0.5 ml blood, vitreous or urine
- Carfentanil-D5 internal standard
- Buffer and SPE extraction
- Dry-down and reconstitute in 100 ul acetonitrile/DIW
- 5 ul analyzed by LC-MS/MS

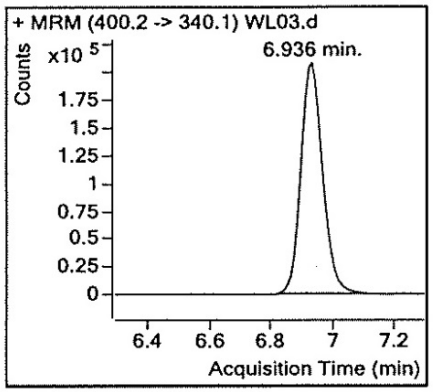
Quantitation by LC-MS/MS

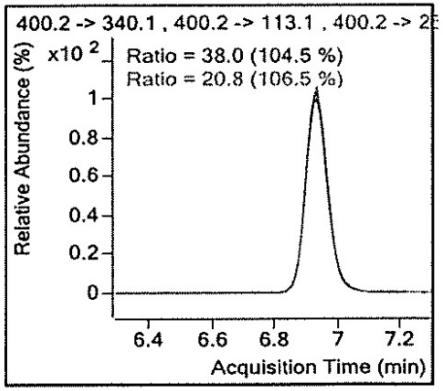
- SPE Extraction based on 0.5 ml blood; 5 ul ext. injected
- LC-MS/MS (6460)
  - Carfentanil-d0: 395.2 and product ions 335.2, 246.1 & 113.2
  - Carfentanil-d5: 400.2 and product ions 340.2, 251.1 & 113.2
- Calibration 0.01 – 2 ng/mL (8 points; each within +/-20% of target): r2 = 0.999
- Control at 0.1 ng/mL (must be within +/-20% of target)
- Spike recovery on case bloods must be 80 - 120% of target



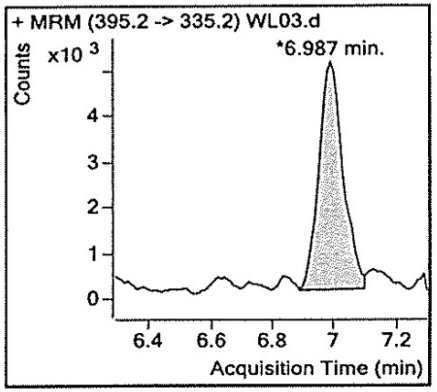
Carfentanil 10 pg/mL Calibrator

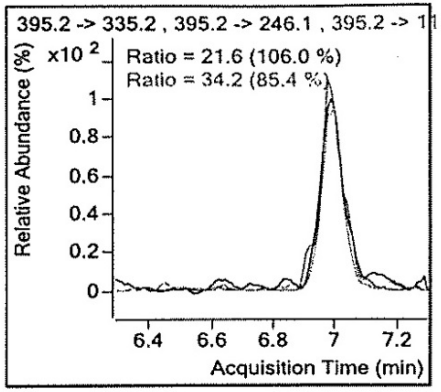
Carfentanil-D5





Carfentanil





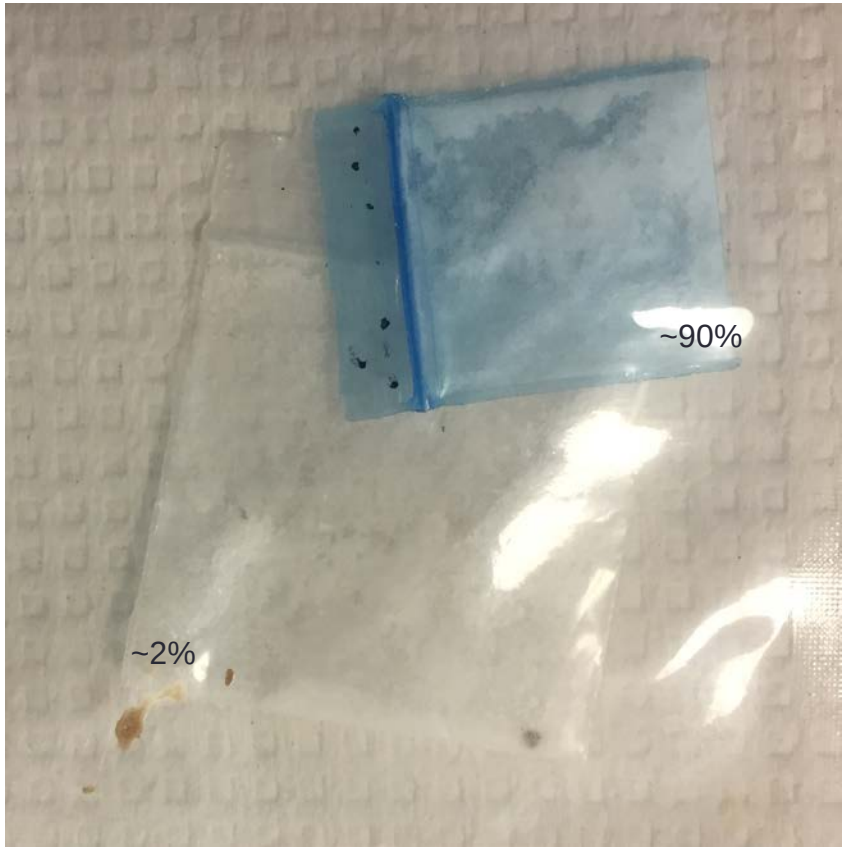
Detecting and identifying new opioids is extremely challenging – what are we doing?

- Seizing and analyzing drug paraphernalia
  - Higher concentrations make for easier detection
  - May provide drug source to help identify new illicit drugs
- Optimizing and/or developing new analytical methods
  - Applying to **purchase more sensitive equipment**
- Information gathering
  - Keeping in touch with colleagues (Canada, USA and elsewhere)
  - Contact with police agencies, Health Canada
  - Reading current scientific literature, news articles
  - Following popular drug related ‘blogs’
  - Looking at ‘research chemicals’ that are being offered

Why is illicit ‘fentanyl’ dangerous?

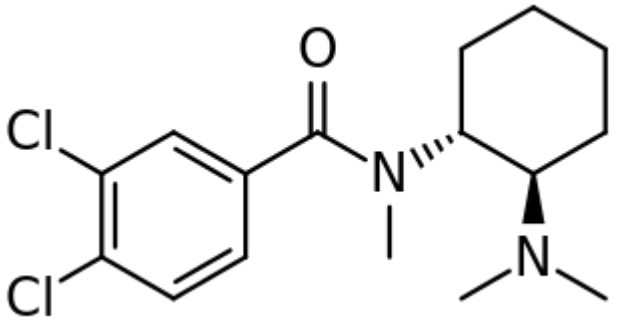
- Drug dealers don’t have good quality control!
- The drug user:
  - Doesn’t know how much drug is in the powder / tablet
  - Doesn’t know whether the amount of drug is consistent from tablet to tablet within the same batch (‘hot spots’)
  - Doesn’t know which drug is in the powder / tablet
  - Many powders contain multiple drugs

You never really know what you are looking at…



Other opioids (e.g. U-opioids)

- U-47700 developed and researched by Upjohn Pharmaceuticals in late 1970s
- Opioid with est. potency of 7.5 times morphine
- Not controlled by CDSA but notice to include
- Also: U-49900, U-51754 *and many others*



U-47700

W-18 – an update

Originally:

- *A series of substituted piperidylidene-2-sulfon(cyan)amide derivatives with potential analgesic agonist or antagonist activity*
  - *Work published in 1981 and patent granted in 1984 (E. Knaus, B. Warren, A. Ondrus)*
- *Dose for 50% inhibition of writhing (mg/kg)*
  - *Morphine 0.038 (x 1)*
  - *W-18 0.0000037 (x 10270)*

Now:

- Nobody is really sure what W-18 does…but we know what it doesn't do.
- Research at University of North Carolina:
  - W-18 is NOT an opioid; not an agonist or antagonist for mu, delta or kappa opioid receptors.
  - W-18 metabolites were also found to be devoid of opioid activity.
  - W-18 did produce atypical burrowing and tunneling activity (into bedding).
  - **PHARMACOLOGY OF W-18 AND W-15** http://biorxiv.org/content/early/2016/07/24/065623

Why is the opioid / fentanyl ‘outbreak’ *different*?

QUESTIONS?