Fentanyl Purity, Potency, & Synthesis

Real-Time Testing of Opioid Drug Products in the United States

WHAT IS FENTANYL?

Fentanyl is a synthetic opioid first synthesized in 1960. Fentanyl is used widely in medicine for the treatment of severe pain. Fentanyl is reported to be 50 to 100 times more potent than morphine. Like other opioids, fentanyl is a central nervous system depressant and in overdose scenarios can lead to a range of adverse effects, including sedation, respiratory depression, and in severe cases fatal overdose. The prevalence of fentanyl in the United States recreational drug supply has continued to increase since the mid-2000s, becoming the most frequently encountered opioid in the United States. Recreational fentanyl (sometimes referred to as illicitly manufactured fentanyl) is the primary synthetic opioid identified in fatal drug overdoses, although there are increasingly reports of fentanyl poly-drug occurrences (e.g., in combination with xylazine, benzodiazepine, stimulants). Recreational fentanyl is commonly ingested through various routes of administration, including injection, smoking, and ingestion. Fentanyl remains a drug of high public health concern among an increasingly volatile drug supply, and its prevalence has thus far only increased despite various countermeasures.

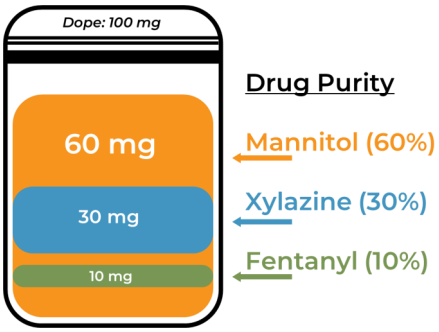

Figure 1: Illustration of drug purity.

WHAT ARE FENTANYL PRECURSORS, INTERMEDIATES, AND BYPRODUCTS?

Fentanyl is a synthetic drug produced via chemical processes and reactions between starting materials or precursors. Fentanyl can be chemically synthesized in different ways (*Figures 5-9*) using a variety of precursors. **Precursors** are defined as the starting molecules used for synthesis. During controlled pharmaceutical synthesis, careful selection of chemical reactions and clean-up processes ensure a correct pathway is followed, flowing through known **intermediates** to high-purity final products (*Figure 4*). However, during clandestine synthesis, it is common that byproducts (or impurities) can appear. **Byproducts** are defined as unwanted molecules produced or left behind from chemical reactions of precursors or with intermediates. Byproducts are not the intended final drug product (e.g., fentanyl). Examples of precursors include 4-ANPP, 4-AP, benzylfentanyl, phenethylchloride, and NPP. Examples of intermediates include 4-ANPP, 4-ANBP, and benzylfentanyl. Examples of byproducts include 4-ANPP, phenethyl-4-ANPP, *N*-propionyl norfentanyl, and acetylfentanyl. To complicate matters, some molecules can be classified as a precursor and/or an intermediate and appear as a suspected byproduct (e.g., 4-ANPP) depending on the synthesis route. Based on currently available data or interpretive assessments, fentanyl precursors, intermediates, and byproducts are largely inactive or retain only very low opioid activity (with the exception of acetylfentanyl), making their presence in drug materials, especially in small quantities, of low pharmacological significance (although true toxicity of these chemicals remains unknown).

WHAT ARE PURITY AND POTENCY?

Purity is defined as the amount or quantity of a specific drug in a material or product. Purity is assessed on an individual drug basis and can be reported as a percent (%) or absolute weight (mg). For example, the purity of a 100 mg powder might be 10% (or 10 mg) for fentanyl, 30% (30 mg) for xylazine, and 60% (60 mg) for mannitol (*Figure 1*). **Potency** is defined by the strength of effects the drug product can have in humans. Potency relates the purity of all pharmacologically active constituents. **Purity vs. potency** is best described by methamphetamine quantitative testing where purity is the amount of methamphetamine in a product and potency is more specifically the amount of *d*-methamphetamine. Herein relating to fentanyl, purity is the amount of fentanyl in a product while the **Potency Index** (*Figure 3*) represents the combined effects of all opioids, including drugs like heroin and *para*-fluorofentanyl. *Potency index* is calculated using relative pharmacological activity (EC_{50}), normalized, and expressed on a scale where a fentanyl powder of 10% purity represents a baseline unit of 1.

HOW IS PURITY DETERMINED?

Purity testing is determined through accurate quantitative analysis using **gas chromatography mass spectrometry (GC-MS)**. An external calibration model is developed using known quantities of drug reference materials to which the sample in question is compared. A defined weight (mg) of the drug product is measured followed by a series of specified dilutions in organic solvents and extraction of drug(s) from matrix (i.e., isolation from unwanted components). A final calculation is performed to determine purity as a percent (%) relative to the initial measured mass of an aliquot (or sub-sample).

HOW DOES PURITY TESTING SUPPORT HARM REDUCTION?

Qualitative fentanyl testing of drug products has been employed for many years in various forms for harm reduction purposes, from the employment of fentanyl test strips and FTIR in the field to GC-MS and LC-MS assays in the laboratory. The results of qualitative testing for fentanyl are useful in certain scenarios; however, it has been observed that qualitative testing alone cannot answer more complex questions about drug products and their impacts (e.g., overdose surges, unexpected adverse effects). It has long been hypothesized that comprehensive, population-level quantitative fentanyl testing would serve as a better assessment of the drug supply – an observation that continues to be assessed and validated in countries outside the United States. Having fentanyl purity and potency data allows public health officials to better understand and assess the drug supply and use outcomes. Our preliminary data show that drug purity can vary between samples marked identically over time (*Figure 2*).

Stamp "X"

June 2022
Fentanyl (9%), Xylazine (24%)
Potency Index: 0.9

June 2022
Fentanyl (9%), Xylazine (22%)
Potency Index: 0.9

June 2022
Fentanyl (10%), Xylazine (22%)
Potency Index: 1.0

June 2022
Fentanyl (10%), Xylazine (21%)
Potency Index: 1.0

July 2022
Fentanyl (21%), Xylazine (19%)
Potency Index: 2.1

July 2022
Fentanyl (15%), Xylazine (19%)
Potency Index: 1.5

July 2022
Fentanyl (9%), Xylazine (45%)
Potency Index: 0.9

Figure 2: Authentic quantitative data from drug products with identical markings (stamp) collected in Philadelphia, Pennsylvania, USA, showcasing fluctuation in purity and potency.

Objectives: A partnership between the Center for Forensic Science Research and Education (CFSRE) and the Philadelphia Department of Public Health (PDPH) has been established to accurately assess the drug supply in Philadelphia, Pennsylvania, USA. This initiative was established as a comprehensive effort examining various drug materials and drug forms, in both qualitative and quantitative fashions. The information and results reported herein represent a subset of the drug supply and not its entirety.

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Fentanyl Purity, Potency, & Synthesis (CONT.)

DRUG PURITY VS. POTENCY INDEX

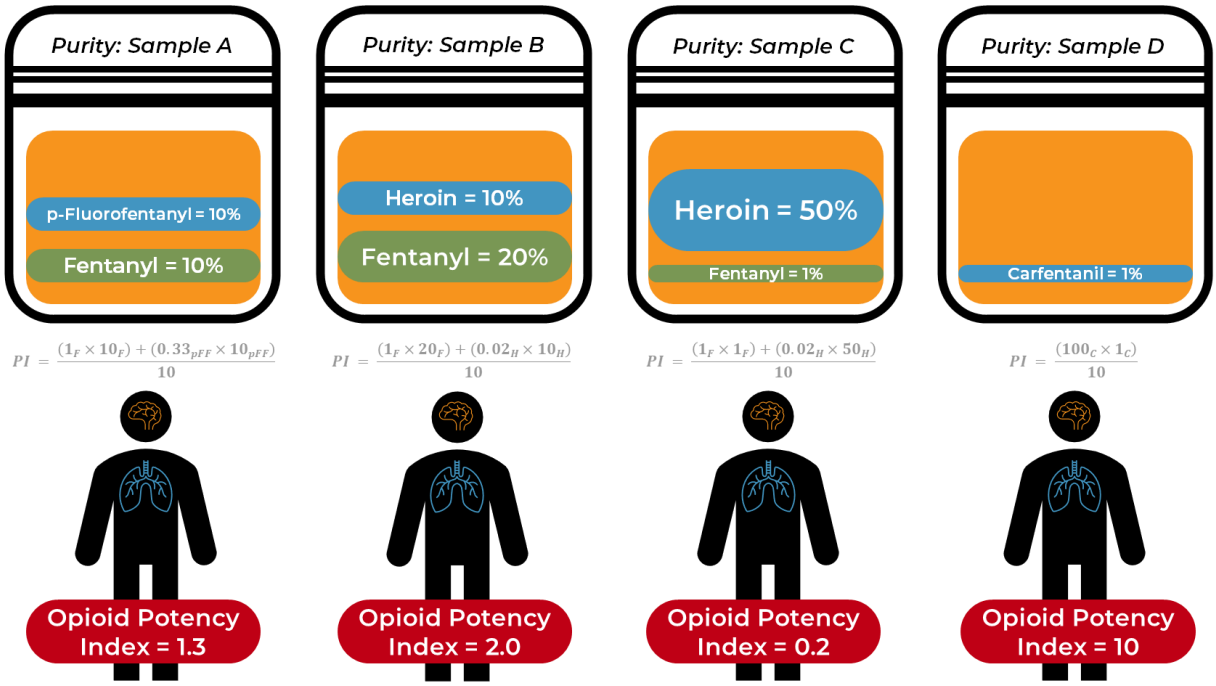
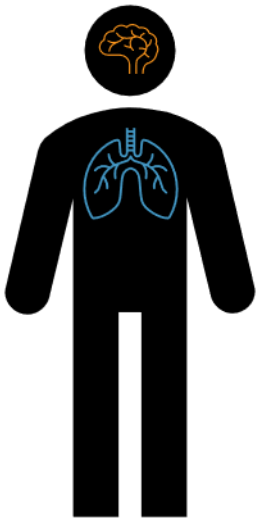


Figure 3: Illustration of drug purity (top) vs. potency index (bottom) with example calculations for opioids commonly encountered in the recreational drug supply.

$$Potency\ Index = \frac{(PF \times Purity) + (PF \times Purity)\ \ldots}{10} \qquad (Eq.1)$$

CALCULATING POTENCY INDEX (PI)

The United States is in the midst of an overdose epidemic which is underlined by poly-drug use. Poly-drug combinations can be rooted in drug products containing, at times, more than one opioid. As quantitative drug purity data become available, scientists and public health officials need a comprehensive yet simple methodology to compare drug products. For this purpose, the **potency index** was developed. *Potency index* is a numeric value that takes into account the amount of a drug present (i.e., purity) and its relative potency, or *potency factor* (e.g., compared to fentanyl). The calculation is the sum of all similar pharmacologically active drugs present and is normalized to a comparator (e.g., fentanyl at 10% purity) for ease of understanding and utility. Potency *index* can be calculated for and applied to other drug classes, but herein is used as a representation of opioids.



Equation 1 can be applied to calculate Opioid Potency Index, where…

- ⇒ PF is the *Potency Factor* compared to fentanyl and is calculated as $EC_{50}^{Fent} / EC_{50}^{Opioid}$ at the mu opioid receptor.
- ⇒ Examples of PFs: Fentanyl = 1, Heroin = 0.02, *para*-Fluorofentanyl = 0.33, *ortho*-Fluorofentanyl = 3, Carfentanil = 100.
- ⇒ *Purity* is the amount of drug in a specified sample and is expressed as a percent (e.g., 10%, 23%).
- ⇒ […] indicates that the numerator should be continued for all drugs (in this case opioids) present in the sample.
- ⇒ The denominator is 10 — a *Normalizing Factor* applied so a sample of 10% fentanyl-only represents a *Potency Index* of 1.
- ⇒ *Potency Index* is reported to one decimal place (e.g., 0.9, 4.6, etc.) until the value eclipses 10.
- ⇒ Tolerance, dose, and other use factors are assumed to be constant at the individual level when assessing *Potency Index*; however, it should be understood that these factors will influence inter-individual outcomes.

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Figure 4: Generic flow of chemicals during drug synthesis. Illustration shows when byproducts may be formed during the synthesis process.

FENTANYL SYNTHESIS PATHWAYS

FIGURE 5: JANSSEN (A)

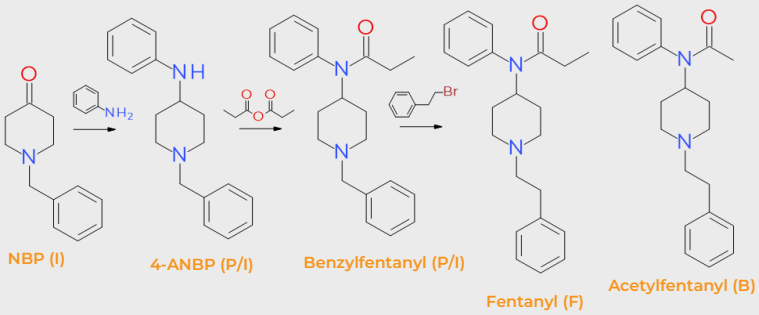


FIGURE 6: SIEGFRIED (B)

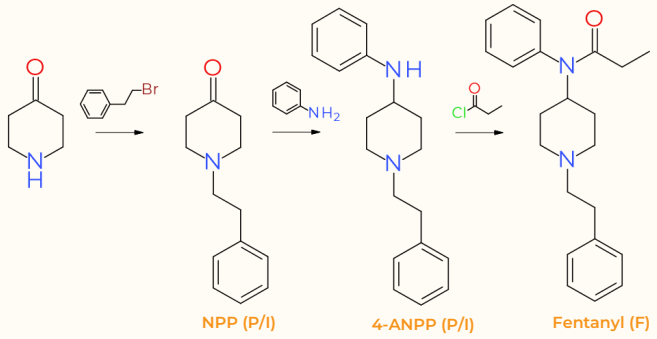


FIGURE 7: GUPTA 1 (C)

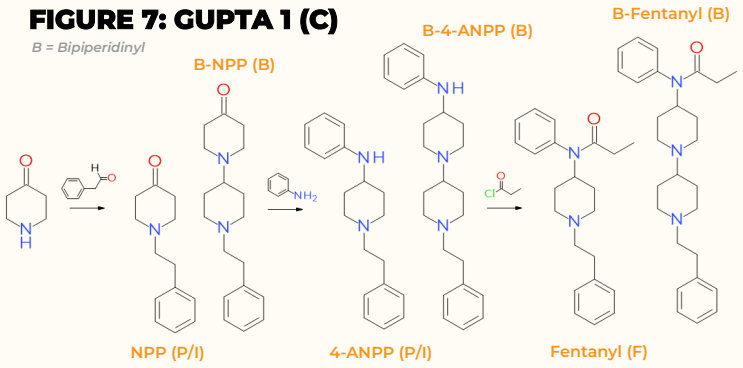


FIGURE 8: GUPTA 2 (D)

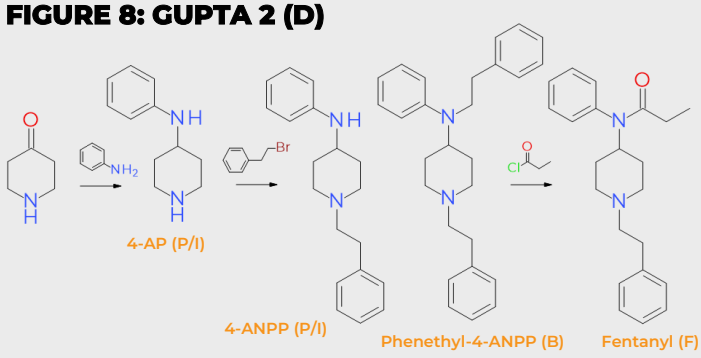
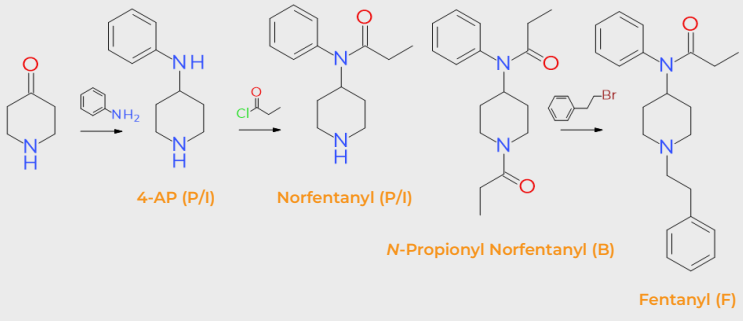


FIGURE 9: GUPTA 3 (E)



Molecule Name	Classification	Pathway Observed
4-ANBP	Precursor / Intermediate	A
4-ANPP	Precursor / Intermediate	B, C, D
4-AP	Precursor / Intermediate	D, E
Acetylfentanyl	Byproduct	A *(Others possible)*
B-4-ANPP	Byproduct	C
Benzylfentanyl	Precursor / Intermediate	A
B-Fentanyl	Byproduct	C
B-NPP	Byproduct	C
NBP	Precursor / Intermediate	A
Norfentanyl	Precursor / Intermediate	E *(A not shown)*
NPP	Precursor / Intermediate	B, C
N-Propionyl Norfentanyl	Byproduct	E
Phenethyl-4-ANPP	Byproduct	D

Note: All precursors, intermediates, and/or byproducts may not be listed for all pathways.

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