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## Treatment of overdose in the synthetic opioid era

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## ABSTRACT

Overdose deaths are often viewed as the leading edge of the opioid epidemic which has gripped the United States over the past two decades (Skolnick, 2018a). This emphasis is perhaps unsurprising because opioid overdose is both the number-one cause of death for individuals between 25 and 64 years old (Dezfulian et al., 2021) and a significant contributor to the decline in average lifespan (Dowell et al., 2017). Exacerbated by the COVID 19 pandemic, it was estimated there were 93,400 drug overdose deaths in the United States during the 12 months ending December 2020, with more than 69,000 (that is, >74%) of these fatalities attributed to opioid overdose (Ahmad et al., 2021). However, the focus on mortality statistics (Ahmad et al., 2021; Shover et al., 2020) tends to obscure the broader medical impact of nonfatal opioid overdose. Analyses of multiple databases indicate that for each opioid-induced fatality, there are between 6.4 and 8.4 non-fatal overdoses, exacting a significant burden on both the individual and society. Over the past 7–8 years, there has been an alarming increase in the misuse of synthetic opioids (“synthetics”), primarily fentanyl and related piperidine-based analogs. Within the past 2–3 years, a structurally unrelated class of high potency synthetics, benzimidazoles exemplified by etonitazene and isotonitazene (“iso”), have also appeared in illicit drug markets (Thompson, 2020; Ujvary et al. 2021). In 2020, it was estimated that over 80% of fatal opioid overdoses in the United States now involve synthetics (Ahmad et al., 2021). The unique physicochemical and pharmacological properties of synthetics described in this review are responsible for both the morbidity and mortality associated with their misuse as well as their widespread availability. This dramatic increase in the misuse of synthetics is often referred to as the “3rd wave” (Pardo et al., 2019; Volkow and Blanco, 2020) of the opioid epidemic. Among the consequences resulting from misuse of these potent opioids is the need for higher doses of the competitive antagonist, naloxone, to reverse an overdose. The development of more effective reversal agents such as those described in this review is an essential component of a tripartite strategy (Volkow and Collins, 2017) to reduce the biopsychosocial impact of opioid misuse in the “synthetic era”.

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## Contents

1. Introduction . . . . .	0
2. Economic impact of opioid overdose . . . . .	0
3. Opioid overdose: effects on the central nervous system . . . . .	0
4. Pharmacological perspectives . . . . .	0
Declaration of Competing Interest . . . . .	0
Acknowledgements . . . . .	0
References . . . . .	0

*Abbreviations:*  $C_{max}$ , maximum plasma concentration; CNS, central nervous system; DEA, Drug Enforcement Administration; ED, emergency department; GPCR, G protein-coupled receptor; IN, intranasal; log P, the logarithm (base 10) of the octanol/water partition coefficient, a measure of a compound's lipid solubility; OUD, opioid use disorder; MOR,  $\mu$  opioid receptor (s); SYNTHETICS, opioids that are neither constituents of (e.g. morphine) nor derived from (e.g. heroin, hydrocodone) the opium poppy;  $t_{1/2}$ , elimination half-life of a drug;  $t_{50}$ , time required for plasma concentration to decrease by 50% following cessation of an infusion;  $T_{max}$ , the time when maximum plasma concentrations are achieved; WCS, Wooden Chest Syndrome.

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## 1. Introduction

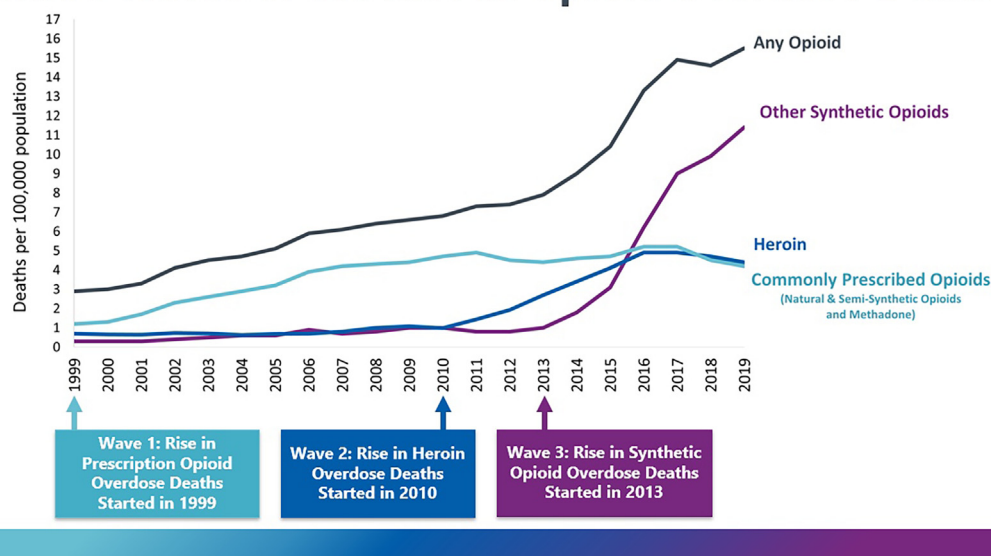
While over 2 million Americans aged 12 or older (that is, ~0.7% of the population) meet DSM-5 criteria for an opioid use disorder (OUD), opioid misuse (defined here as use outside of prescribed parameters) is far more prevalent. In 2018, more than 10.3 million people misused opioids in the past year (Substance Abuse and Mental Health Services Administration, 2019). Both OUD and opioid misuse have serious biopsychosocial consequences, ranging from the potential for involvement in the criminal justice system to an increased risk of contracting hepatitis C and HIV through injection drug use (Stein, 1999). Among these, overdose is a frequent, if not inevitable consequence of illicit opioid use. Studies conducted in Australia during the 1990s indicate that between 50 and 70% of intravenous heroin users experienced a non-fatal overdose, with 20–30% of victims reporting the overdose occurred in the preceding 12 months (Warner-Smith, Darke, & Day, 2002; Warner-Smith, Darke, Lynskey, & Hall, 2001). More recent studies provide compelling evidence that individuals who survive a nonlethal opioid overdose are at higher risk of a subsequent overdose both within 30 days and over the next 12 months following the initial event. For example, in a retrospective study of over 4100 patients who survived an overdose, Suffoletto and Ziegler (2020) reported ~15% of patients had a repeat overdose, with 29% occurring within the first 30 days (Suffoletto & Ziegler, 2020). Both short and long-term mortality for individuals suffering a non-fatal overdose is high: in a cohort of over 11,500 patients, 5.5% died 1 year, with ~20% of these fatalities occurring within 30 days (Weiner, Baker, Bernson, & Schuur, 2020). Moreover, during the first 12 months following an overdose, estimates of the all cause and drug-use associated diseases mortality rates were 24.2 and 132-fold higher, respectively, than a demographically matched general population (Olfson et al., 2018). Given the increased availability of illicit synthetics (Drug Enforcement Administration, 2021; Reuter, Pardo, & Jirka, 2021) and the hazards associated with misuse of these potent opioids, it is likely the number of individuals who experience an overdose will continue to rise.

The Centers for Disease Control (CDC) estimated there have been more than 500,000 opioid overdose deaths over the past two decades

(<https://www.cdc.gov/drugoverdose/epidemic/index.html>) [Fig. 1] with more than 68,800 reported during the 12 months ending December 2020 (Ahmad, Rossen, & Sutton, 2021). Synthetics such as fentanyl are now linked to >80% of these deaths. While public attention remains focused on the lethal effects of opioids, for every overdose death, there are many more non-fatal overdoses exacting a toll on both the victim and society. In 2017, more than 305,000 emergency department (ED) visits involved opioids (Vivolo-Kantor et al., 2020); by comparison, the number of opioid overdose deaths that year was estimated to be 47,500 (Ahmad et al., 2021), a ratio of 6.4:1. Another recent (2019) estimate puts the number of ED, inpatient, and other care settings related to opioid overdose at over 430,000 (<https://revcycleintelligence.com/news/opioid-overdose-care-totals-1.94b-in-annual-hospital-costs>) with overdose deaths estimated at 51,032 for that year (Ahmad et al., 2021), a ratio of ~8.4:1.

The impact of the opioid epidemic varies widely across states (<https://www.cdc.gov/nchs/data-visualization/drug-poisoning-mortality/index.htm>; Shover et al., 2020). Nonetheless, available data from individual states and/or regions provides additional insight into the incidence of nonlethal and lethal overdoses. For example, data from Arizona estimated 9496 overdose deaths between June 2017 and May 2021 and 69,716 suspected opioid overdoses (<https://www.azdhs.gov/prevention/womens-childrens-health/injury-prevention/opioid-prevention/index.php>), or approximately 7.3 nonfatal overdoses per fatality. A similar ratio (~7.4) of opioid related-ED visits to fatalities was obtained in a 2016 sample with a database of ~2.3 million individuals obtained from 4 large Maryland databases (Saloner et al., 2020). Data from these two states fall within estimates of the ratio of non-fatal to fatal overdoses gleaned from national surveys. Based on these data, the number of non-fatal overdoses in the United States through December 2020 is estimated to range between 440,300–577,900. These estimates do not fully capture the increase in opioid overdoses during the COVID-19 pandemic: there has been a 32% increase in opioid-related emergency department visits compared to pre-pandemic values (Holland et al., 2021). Furthermore, hospital-based data are likely to underestimate the number of non-fatal overdoses because many victims rescued by first responders (including police,

## Three Waves of the Rise in Opioid Overdose Deaths



**Fig. 1.** Opioid overdose deaths over the past 2 decades: three “waves” of the opioid epidemic. Prescription opioids such as oxycodone were primarily responsible for the dramatic increase in overdose deaths during the first decade of this millennium. Efforts to add abuse deterrent features to prescription opioids was followed by a spike in heroin related overdose deaths. Synthetic opioids, primarily fentanyl and fentanyl analogs, have driven the so-called “third wave” of opioid-related fatalities. This modified figure is from the National Vital Statistics System Mortality File (<https://www.cdc.gov/drugoverdose/images/3-waves-2019.PNG>).

emergency medical services, and the victim's friends and family) refuse subsequent treatment and are lost to follow up.

## 2. Economic impact of opioid overdose

The estimated annual hospital costs (2017–18) associated with treating patients who experienced an opioid overdose was ~\$1.94 billion, with over \$632 million in ED costs alone (<https://revcycleintelligence.com/news/opioid-overdose-care-totals-1.94b-in-annual-hospital-costs>). About 47% of patients experiencing an opioid overdose were treated and released (at an average cost of \$504), whilst the remaining 53% were treated and admitted, with an associated cost of \$11,731. Among those patients admitted, about 40% experienced organ failure, requiring intensive care with additional costs averaging \$20,500. These estimates do not consider the costs associated with treatment provided by first responders (e.g., emergency medical services; EMS), which can range between \$1600–3000 per incident depending on geographical location (B. Manning, personal communication). Extrapolating hospital cost data indicates that all in costs of medical services directly associated with opioid overdose adds about \$11.3 billion to the healthcare system annually. (<https://www.premierinc.com/newsroom/press-releases/opioid-overdoses-costing-u-s-hospitals-an-estimated-11-billion-annually>). However, estimates of medical costs related to opioid overdose do not fully reflect either its economic or societal impact (Dowell et al., 2017). Florence, Luo, and Rice (2021) have modelled the economic burden of opioid use disorder and fatal opioid overdose, estimated at \$1.02 trillion in 2017. This estimate includes costs for health care, substance abuse treatment, criminal justice, and lost productivity. Most of these costs, estimated at over \$549 billion, are associated with lost productivity and the value of statistical life lost. Although this a contemporaneous study, the data do not capture the current economic burden of opioid overdose deaths because of the ~33% increase in the reported number of fatal overdoses between July 2017 and July 2020 (Ahmad et al., 2021).

## 3. Opioid overdose: effects on the central nervous system

The central nervous system (CNS) plays a preeminent role in opioid overdose not only because it drives respiration, but also because neurons are particularly vulnerable to hypoxic damage resulting from depressed respiration. A more sustained or profound hypoxia will also affect other CNS structures including epithelial cells of the choroid plexus and glia.

Converging lines of evidence indicate that activation of MOR in neurons of the pre-Bötzing complex located in the ventrolateral medulla and the parabrachial complex (which includes the Kölliker-Fuse nucleus) located in the dorsolateral pons, drive opioid-induced respiratory depression (Bachmutsky, Wei, Kish, & Yackle, 2020; Dutschmann & Dick, 2012; Liu et al., 2021; Montandon & Horner, 2014; Smith, Ellenberger, Ballanyi, Richter, & Feldman, 1991). Respiratory depression is responsible for opioid-induced fatalities as well as the short- and long-term morbidities linked to a non-fatal overdose (Boyer, 2012; Zibbell, Howard, Clarke, Ferrell, & Karon, 2019), including brain injuries produced by hypoxia and in more severe cases, anoxia. In broad terms, tissue damage resulting from an opioid overdose is related to the severity and duration of respiratory depression.

During an opioid overdose, a depressed respiratory rate reduces the partial pressure of oxygen in the blood (hypoxemia) resulting in the victim experiencing a reduction in tissue levels of oxygen (hypoxia). Reductions in respiratory rate and the potential for resulting hypoxic damage will depend upon the physiologic (e.g., pre-existing conditions like COPD that affect pulmonary function), pharmacokinetic (e.g., the half-life of the opioid(s) taken) and pharmacodynamic (e.g., potency and quantity of the opioid(s) taken) factors attending the overdose

(Table 1). Hypoxic brain damage can result in multiple pathologies including seizures, temporary motor paralysis, coma, and stroke. In addition, mental disorientation, an amnesic syndrome, ataxia, gait disturbances, paraplegia, catatonia, reduced reaction time, and diminished motor skills and physical functioning have all been linked to hypoxic brain injuries following a nonfatal opioid overdose. (Barash, Sommerville, & DeMaria, 2017; Sommerville et al., 2017; Zibbell et al., 2019). There are also multiple reports in the clinical literature of a demyelinating syndrome (delayed post-hypoxic leukoencephalopathy) caused by conditions which produce sustained oxygen deficiency, including opioid overdose. While the diagnosis is relatively rare, following an apparent recovery, there are a range of neuropsychiatric symptoms typically manifesting 1–2 weeks after the hypoxic insult including disorientation, attention and memory deficits, hyperreflexia, parkinsonism and in more severe cases, catatonia and psychosis (Betts, Ritter, Kubal, 2012; Salazar & Dubow, 2012; Zamora et al., 2015). The prognosis is variable, and the literature specifically addressing opioid overdose consists of a handful of case studies (e.g., Salazar & Dubow, 2012; Zamora et al., 2015).

In a rare prospective study of overdose victims who were subsequently discharged from hospital following overdose with central nervous system depressants (including antipsychotics, benzodiazepines and opioids), Dassanayake et al. (2012) reported significant impairments in multiple cognitive domains considered important to daily functioning such as visuomotor skills, executive functioning and planning, working memory, and impulsivity and decision making compared to patients admitted for a non-CNS depressant related drug overdose (e.g. acetaminophen, SSRIs, SNRIs). While neither the number of opioid overdose patients nor the opioids taken by this cohort were reported, these deficits were described when the patients were deemed eligible for discharge: ~85% of patients were tested within 48 h of presumed drug exposure. There was no subsequent follow up of this cohort to determine the duration of these cognitive deficits.

While opioid overdose is often portrayed as a binary (i.e., fatal/non-fatal) event, the prognosis following a non-fatal opioid overdose is highly variable, with outcomes ranging from an unpleasant experience to long-term physical and mental disability. Among the factors which shape the victim's prognosis are the type, quantity, pharmacokinetic profile, and route of administration of the opioid(s) responsible for the overdose, the presence of other compounds (e.g. benzodiazepines, alcohol) which can act synergistically with opioids to depress respiration, the degree of tolerance the victim may have developed through prior opioid exposure, the victim's general health, and the interval between opioid exposure and attempts at intervention (Table 1). Variations across these factors result in what is essentially a unique profile attending every overdose, with the duration and extent of hypoxemia the primary determinant of a victim's prognosis. Reduced to its simplest terms, the longer an overdose victim remains hypoxemic, the less favorable the prognosis. Conversely, the ability to rapidly restore normal breathing favors a successful outcome.

Absent intervention, a sustained period of hypoxia (or in the extreme, respiratory arrest resulting in anoxia) can produce diffuse brain damage and ultimately, brain death. Even a brief period of anoxia (~ 5 min.) can produce neuron death, potentially resulting in enduring damage. The sustained cerebral hypoxia and associated metabolic changes (e.g., increases in brain lactate, severe acidosis) resulting from an opioid overdose contributes to the observation that cerebral edema and brain death are more commonly observed following cardiac arrest associated with opioid overdose compared to other forms of cardiac arrest (Dezfulian et al., 2021). The opioid epidemic has prompted a new terminology, toxic brain injury, championed by the Brain Injury Association of America describing the type of brain injury produced following a non-lethal opioid overdose (<https://www.biausa.org/public-affairs/media/the-solution-to-opioids-is-treatment>).

**Table 1**  
An opioid overdose primer: factors contributing to victim outcomes.

a. Opioid(s) responsible for the overdose
<ul style="list-style-type: none"> <li>Differences among opioids in affinities at MOR (e.g., Volpe et al., 2011; Burns et al., 2018), in activating post-receptor signaling pathways (He et al., 2021; Vandeputte et al., 2021) and in their physicochemical properties all contribute to the potential for misuse resulting in an overdose. Currently, about 80% of overdose fatalities involve a synthetic such as fentanyl (Ahmad et al., 2021). Multiple opioids are often involved in an overdose (for example, heroin is frequently “adulterated” with synthetics) which can complicate overdose management because of a unique pharmacokinetic profile created by two or more opioids as well as the potential for a synergistic effect on brain hypoxia (Solis, Cameron-Burr, &amp; Kiyatkin, 2017). The opioid(s) responsible for an overdose is only one factor which determines the dose of naloxone, a competitive MOR antagonist, required for an effective reversal. In a small sample of individuals who survived a non-lethal overdose, the dose of naloxone required for reversal was not associated with blood fentanyl concentrations (Krotulski et al., 2021), underscoring the potential contribution of other factors described here in determining victim outcomes.</li> </ul>
b. Quantity of opioid(s)
<ul style="list-style-type: none"> <li>Based on blood and serum analyses, the quantity of opioid(s) responsible for a lethal overdose can vary by more than two orders of magnitude (Martin et al., 2006; Sutter et al., 2017; Thompson et al., 2007). Quantity, together with other factors (including the type of opioid(s) and route of administration) determine the amount and speed at which opioids enter the CNS, critical factors in a victim’s prognosis and the window of opportunity to effect a rescue.</li> </ul>
c. Route of administration
<ul style="list-style-type: none"> <li>Both injection and insufflation result in rapid delivery of opioid to the CNS relative to oral administration. Because of the more intense rewarding effects produced by injection and insufflation, these are the preferred routes of administration for misuse (Volkow &amp; McLellan, 2016). This rapid delivery of opioid to the CNS also reduces the window for successful intervention in an overdose relative to oral administration.</li> </ul>
d. Pharmacokinetic profile
<ul style="list-style-type: none"> <li>The pharmacokinetic profile of an opioid is determined by its physicochemical properties, route of administration, and the potential for individual differences in its metabolism. The physicochemical characteristics of an opioid affecting pharmacokinetics can be exemplified by the very high lipophilicity (log P) of synthetics, which results in a rapid uptake by brain and other lipid-rich tissues compared to opiates like morphine. In an overdose, the route of administration can have a nuanced effect on pharmacokinetic profile. For example, following an oral overdose, the inhibitory effect of opioids on gastrointestinal motility may result in delayed and erratic absorption. Injection and insufflation of pulverized tablets and powders can also produce highly variable absorption. Metabolic differences among individuals, often the result of genetic differences in the activities of cytochrome P-450 isozymes (such as CYP3A4 and CYP2D6) contribute to variability in the pharmacokinetics of opioids such as buprenorphine, methadone, and fentanyl (Burns et al., 2018; Ferrari, Coccia, Bertolina, &amp; Sternieri, 2004). In an overdose, this may be further complicated by large quantities of opioids which can saturate drug metabolizing enzymes (Boyer, 2012). Genetic differences as well as other drugs which can modulate (i.e., either induce or inhibit) the activity of drug metabolizing enzymes can result in an opioid overdose as well as complicate its management. There is a greater risk for re-narcotization (relapse) following a rescue with naloxone (<math>t_{1/2}</math> 1–2 h) if opioids with long plasma <math>t_{1/2}</math> values (e.g., methadone, buprenorphine, fentanyl) are involved in the overdose as concentrations of the rescue agent fall more rapidly than the opioid.</li> </ul>
e. Other non-opioids involved in the overdose
<ul style="list-style-type: none"> <li>CNS depressants such as benzodiazepines and alcohol can act synergistically to depress respiration. The risk of a lethal overdose increases (adjusted hazard ratio: 3.86 [95% CI: 3.49–4.26] in patients concurrently prescribed opioids and benzodiazepines (Park, Saitz, Ganoczy, Ilgen, &amp; Bohnert, 2015). Polydrug use appears to be a common phenomenon in opioid overdose. Synthetic opioids have been identified in ~40% of overdose deaths attributed to cocaine. In Massachusetts, methamphetamine was present in 34% of opioid-related fatalities. In a sample of 20 non-lethal overdose victims (drawn from an urban, level one trauma center), fentanyl was present in 95% of the blood samples. Despite the prevalence of fentanyl in this sample, other opioids (carfentanil, acetylfentanyl, buprenorphine, heroin and heroin metabolites), cocaine, methamphetamine, benzodiazepines, and antidepressants were also identified (Krotulski et al., 2021). This increasing co-use of opioids and stimulants has been referred to as the ‘fourth wave’ of the opioid epidemic (Ciccarone, 2021). Both stimulants (cocaine, methamphetamine) and benzodiazepines have been identified in counterfeit pills containing synthetics (Drug Enforcement Administration, 2021)</li> </ul>
f. Tolerance
<ul style="list-style-type: none"> <li>An overdose victim may have developed tolerance through prior opioid exposure, resulting in the ability to misuse larger quantities of opioids. Tolerance to opioids develops with repeated administration. However, tolerance to the respiratory depressant effects appears to develop more slowly than, for example, to the analgesic and euphorogenic actions which may put experienced opioid users at higher risk of overdose (Hill et al., 2016, White &amp; Irvine, 1999).</li> </ul>
g. General health of victim
<ul style="list-style-type: none"> <li>A range of pre-existing conditions including sleep apnea, chronic obstructive pulmonary disease, morbid obesity, and liver disease can increase the risk of morbidity and mortality in an overdose (Boyer, 2012; Dahan et al., 2010).</li> </ul>
h. Interval between overdose and intervention
<ul style="list-style-type: none"> <li>The combinations resulting from variations in these factors produce what is essentially a unique set of circumstances attending every opioid overdose. Because respiratory depression is the primary driver of the hypoxic, and in the extreme, anoxic tissue damage resulting from overdose, the longer the interval between the appearance of symptoms (typically respiratory depression, miosis, and stupor) (Boyer, 2012) and attempts at intervention, the worse the victim’s prognosis. Conversely, the ability to rapidly restore normal respiration will improve the victim’s prognosis.</li> </ul>

## 4. Pharmacological perspectives

### 4.1. Synthetic opioids, the 3rd wave of the opioid epidemic

Over the past 6–7 years, the misuse of synthetic opioids (“synthetics”) such as fentanyl has been the primary driver behind the marked rise (Fig. 1) in opioid overdose deaths (Ahmad et al., 2021). The increased availability of illicit synthetics is driven by economics, shaped in large part by the chemistry and pharmacology of these molecules. Thus, synthetics are far less expensive to produce than opiates (i.e., opium-derived molecules such as morphine and semi-synthetic opiates such as heroin and oxycodone). With appropriate, relatively simple starting materials, multiple fentanyl analogs can be synthesized in high yield with a three-step process (Katselou, Papoutsis, Nikolaou, Spiliopoulou, & Athanaselis, 2016; Valdez, Leif, & Mayer, 2014). Similarly, benzimidazoles such as etonitazene can be synthesized in 3–4 steps with simple starting materials (Ujvary et al., 2021). Based on ease of manufacture, synthetics present fewer supply-side issues than

opiates: the need to cultivate, harvest, and process opium is eliminated, as are attendant problems ranging from drought to political turmoil which can disrupt supply. As a result, the estimated cost of fentanyl obtained on the dark web is estimated to be 5–10% that of heroin (Frank and Pollack, 2017; Mars, Rosenblum, & Ciccarone, 2019). At a 10–20-fold lower cost and a potency ~50-fold higher than heroin (Baumann, Kopajtic, & Madras, 2018; Burns, Cunningham, & Mercer, 2018), there is a compelling economic incentive for drug dealers to either adulterate or substitute a synthetic such as fentanyl for heroin (Mars et al., 2019; Suzuki & El-Haddad, 2017). Counterfeit tablets adulterated with synthetics but labelled as benzodiazepines, opiates, or stimulants pose a significant hazard which is compounded by their high potencies and the inevitable dose-to-dose variability (Sutter et al., 2017; Winter, Schecter, & Snow, 2021) of illicitly manufactured and distributed drugs.

The simple, 4-anilidopiperidine-based core of fentanyl-based synthetics is also highly mutable. This is evidenced by over 1400 fentanyl derivatives described in the patent and scientific literature (Misailidi et al., 2018) with the chemical space of this pharmacophore not fully

explored. Only a small fraction of these compounds has been characterized pharmacologically (Misailidi et al., 2018), and multiple fentanyl analogs available on the dark web have not been examined in detail (Burns et al., 2018; Suzuki & El-Haddad, 2017). Nonetheless, multiple fentanyl analogs are more potent than the parent, with carfentanil, used as a large animal tranquilizer, reported to be ~100-fold more potent with an estimated lethal dose of 20 µg in a non-tolerant individual (Burns et al., 2018; Ringuette, Spock, Lindsley, & Bender, 2020). The sheer number of potential fentanyl analogs presents a significant impediment at attempts to limit distribution, and while fentanyl remains the predominant illicit synthetic opioid, multiple fentanyl analogs (e.g. 3-methylfentanyl, 4-fluoroisobutyrylfentanyl, acetylfentanyl, and carfentanil) have been identified by the DEA (Fig. 2).

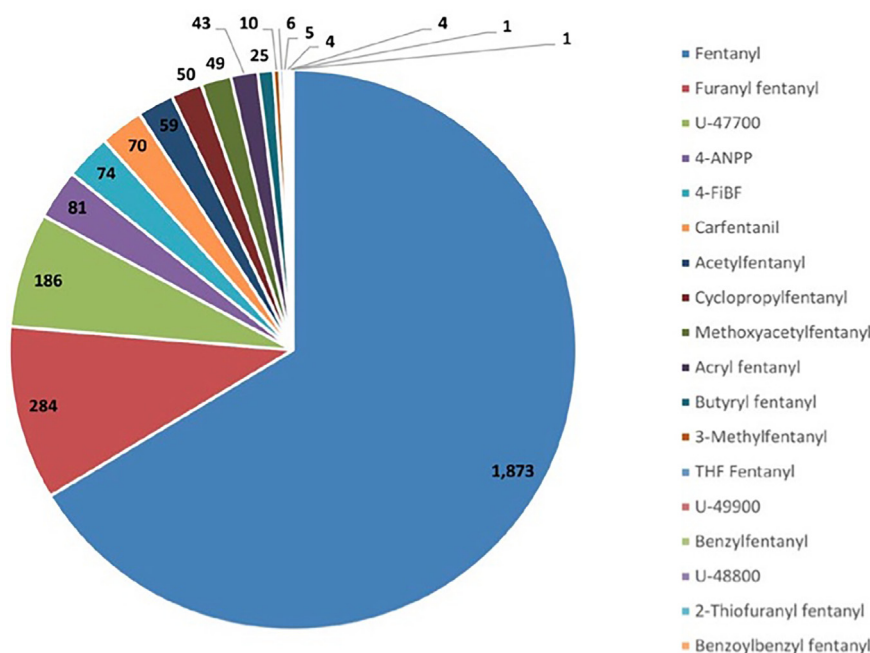
Seizures of 3-methylfentanyl by law enforcement bring into focus some of the risks inherent in the misuse of illicit synthetics. The presence of two centers of asymmetry results in 4 possible stereoisomers of 3-methylfentanyl: a diastereomeric pair (cis/trans) and the optical isomers (+/-) of each. The (+)-cis-3-methyl enantiomer binds to MOR with ultrahigh (i.e., low pM) affinity (Rothman et al., 1991) and reported to be >100-fold more potent than the (-)-cis-3-methyl enantiomer and 19-fold more potent than fentanyl, respectively, as an analgesic following IV administration to rats (Van Bever, Niemegeers, & Janssen, 1974). The synthesis of illicit 3-methylfentanyl would be likely to yield a mixture of the racemic cis- and trans- diastereomers, with (±)-cis-3-methylfentanyl >5-fold more potent than the (±)-trans-3-methyl diastereomer as an analgesic (Van Bever et al., 1974). Because the ratio of cis- and trans- diastereomers is dependent on the synthetic route (Kim et al., 1989; Van Bever et al., 1974), introducing even modest changes to synthetic conditions could result in significant batch to batch variability in the potency of 3-methylfentanyl.

Most illicit synthetics currently originate in China and Mexico (Pardo et al., 2019), but given the relative ease of synthesis, success in reducing illicit imports would likely result in a shift to domestic production. The high potencies of synthetics also facilitate transport and distribution relative to both prescription opioids and heroin: a 20 g tin of

fentanyl is the 'equivalent' of about 1 kg of heroin; the same quantity of carfentanil is equivalent to ~100 kg of heroin. The dangers associated with the misuse of synthetics in this third wave of the opioid epidemic (Dezfulian et al., 2021; Volkow & Blanco, 2020) are substantially greater than those posed by either prescription opioids or heroin. The high potency of synthetics coupled with the inevitable dose-to-dose variability (Sutter et al., 2017; Winter et al., 2021) of illicitly manufactured and distributed drugs certainly contribute to these risks, but factors beyond high affinity at MOR magnify the risk for accidental overdose and poor health outcomes.

Thus, when assayed in broken cell (membrane fragment) preparations using radioligand binding techniques, fentanyl analogs like carfentanil and lofentanyl bind to MOR with ultra-high (pM) affinities (Burns et al., 2018; Rothman et al., 1991). However, the apparent affinity of fentanyl (~1 nM) is comparable to morphine (Rothman et al., 1991; Volpe et al., 2011; Burns et al., 2018, Ringuette et al., 2020; Eshleman et al., 2020; Kelly et al., 2021), which does not reflect the 50–100-fold potency difference between these compounds observed in multiple *in vivo* assays (Burns et al., 2018; Kelly et al., 2021; Suzuki & El-Haddad, 2017). When directly injected into the central nervous system of rats, fentanyl is about one order of magnitude more potent than morphine in depressing respiration as measured by plethysmography, and 10–20-fold more potent than heroin at depressing brain oxygen concentrations (Kiyatkin, 2019). Moreover, data obtained in rodents substantially underestimates the dangers posed by synthetics to primates (Feasel, Lawrence, Kristovich, Wohlfarth, & Huestis, 2018). Thus, early studies in rats demonstrated that despite very high potencies as analgesics and anesthetics, the therapeutic indices (defined as lethal dose<sub>50</sub>/analgesic dose<sub>50</sub>) of fentanyl and several of its analogs, including carfentanil, were orders of magnitude higher than morphine (Mather, 1983); by contrast, the therapeutic index of carfentanil in primates is ~10 (Feasel et al., 2018).

Multiple factors may contribute to the high potencies and dangers posed by synthetics such as fentanyl and its analogs relative to their *in vitro* affinities at MOR. For example, fentanyl has been reported to exhibit a preferential activation (bias) of β-arrestin pathways following



**Fig. 2.** Identification of fentanyl and related synthetics by the DEA in calendar year 2017. Fentanyl accounted for about two-thirds (1873) of all synthetics seized and analyzed (2825) by the DEA. However, at least 18 other synthetics, most structurally related to fentanyl, were also identified. Among the other synthetics seized and identified, furanyl fentanyl was identified in about 10%, U-447700 (a non-fentanyl benzamide derivative) in about 6.6%, 4-ANPP (a fentanyl precursor) in about 2.9%, 4-FiBF (4-fluoroisobutyrylfentanyl) in about 2.6%, carfentanil in about 2.5%, acetylfentanyl in about 2%, cyclopropylfentanyl in about 1.8%, methoxyacetylfentanyl in about 1.8%, acryl fentanyl in about 1.5%, and the remaining compounds in <1% of samples. While fentanyl remains the principal synthetic seized and identified by the DEA, data from the National Forensic Laboratory Information System (NFFLIS) demonstrates substantial year-over-year differences in the synthetics identified. Source: DEA (<https://www.dea.gov/sites/default/files/2018-11/DIR-032-18%202018%20NFTA%20%5Bfinal%5D%20low%20resolution%20of%20110218notcomp.pdf>).

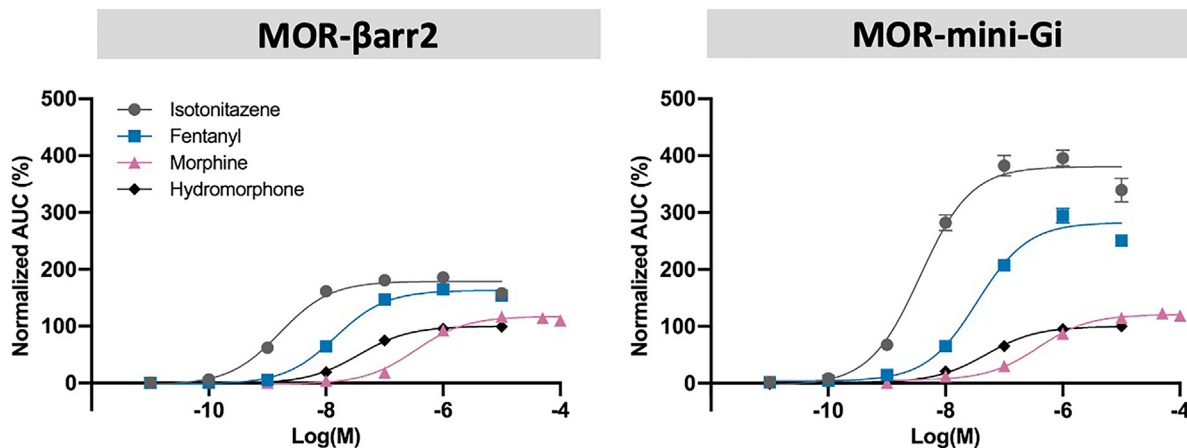
binding to MOR, and this has been hypothesized to contribute to its potency as a respiratory depressant relative to opiates like morphine (Schmid et al., 2017). However, more recent studies have questioned the role of  $\beta$ -arrestin dependent signaling in the respiratory depressant effects of opioids and more broadly, the relationship between ligand bias at the  $\mu$  opioid receptor and pharmacological activity (Kliwer et al., 2020; Gillis et al., 2020; He et al., 2021). Nonetheless, in cell-based assays synthetic opioids including fentanyl and isotonitazene are both more potent and efficacious than natural (morphine) and semi-synthetic (hydromorphone) opiates (He et al., 2021; Kelly et al., 2021; Vandeputte et al., 2021) at recruiting multiple intracellular signaling pathways mediating pharmacological actions of opioids (Fig. 3). One hypothesis which may explain, at least in part, affinity, efficacy, and potency differences between morphine and fentanyl observed in broken cell and cell-based (and in vivo) assays may be related to the high lipophilicity of synthetics (see next paragraph). High lipophilicity could produce a more effective partitioning of a synthetic like fentanyl into the cell membrane, resulting in higher concentrations within the microenvironment of the MOR relative to less lipophilic molecules like morphine (Kelly et al., 2021). Also consistent with the hypothesis that differences in post-receptor signaling and/or membrane partitioning could contribute to the high potencies of synthetics, molecular dynamic simulations of MOR indicate morphine preferentially activates transmembrane helices 3 and 5, whilst fentanyl preferentially activates helices 6 and 7 which are associated with GPCR activation (Ricarte, Dalton, & Giraldo, 2021).

The high lipophilicity of synthetics relative to opiates is a significant contributor to the increased risk for overdose. For example, fentanyl (log P 4.28) is >1000-fold more lipophilic than morphine (log P 1.07) (Burns et al., 2018) and benzimidazoles exemplified by etonitazene and its isopropyl analog (isotonitazene) exhibit similar high lipophilicities, with calculated log P values in the range of 4.1–5.1 (Ujvary et al., 2021). High lipophilicity results in rapid penetration into the CNS, a valued clinical attribute in an analgesic/anesthetic which has resulted in the widespread use of synthetics like fentanyl and remifentanyl in surgical procedures. The subjective effects of fentanyl and fentanyl analogs are manifested within one circulation time after IV administration, and clinically relevant analgesia/anesthesia and respiratory depression produced within minutes (Suzuki & El-Haddad, 2017). However, this very rapid entry into the CNS effectively compresses the window of opportunity for successful intervention in an overdose (Table 1). The difference in onset between synthetics and

opiates is also readily demonstrable in preclinical models: for example, following IV injection in rats, the latency (i.e., the interval between injection and first significant reduction) of fentanyl to reduce brain oxygen concentrations measured using an oxygen sensor coupled to high speed amperometry is twice as fast (32 s) as either oxycodone (65 s) or morphine (70 s) (Kiyatkin, 2019). Using plethysmography in mice, Hill, Santhakumar, Dewey, Kelly, and Henderson (2020) reported the rate of onset for fentanyl to depress minute volume ( $t_{1/2}$  0.54 min) was approximately 3- and 9-fold faster than heroin ( $t_{1/2}$  1.70 min) and morphine ( $t_{1/2}$  4.64 min), respectively, following IV injection.

The high lipophilicity of synthetics can be especially insidious in an overdose because there is an initial rapid decline in plasma concentrations ( $t_{50}$ ) as the synthetic opioid partitions into the CNS and other lipid-rich tissues. This may complicate the management of overdose using naloxone ( $t_{1/2}$  1–2 h) [Ryan & Dunne, 2018] as plasma concentrations of the rescue agent fall more rapidly than the opioid (s) responsible for the overdose, with the potential for re-narcotization (Dahan, Aarts, & Smith, 2010). For example, reported  $t_{50}$  values for sufentanil and fentanyl are 69 and 92 min., respectively, whilst the corresponding  $t_{1/2}$  values are >6 h and 7.5 h (Ahonen et al., 2000). Multiple clinical studies (Holley & Van Steenis, 1988; Scott & Stanski, 1987) are consistent with a  $t_{1/2}$  of fentanyl in the 8 h range. While there is limited human data on the pharmacokinetic profile of carfentanil (Zawilska, Kuczynska, Kosmal, Markiewicz, & Adamowicz, 2021), based on a case study  $t_{1/2}$  values for carfentanil and norcarfentanil (an active metabolite) were estimated to be 5.7 and 11.8 h, respectively (Uddayasankar, Lee, Oleschuk, Eschun, & Ariano, 2018). A subsequent slow redistribution from lipid-rich tissues back to plasma has been hypothesized to contribute to the reported  $t_{1/2}$  values and sustained respiratory depressant effects of synthetics (Suzuki & El-Haddad, 2017).

Illicit synthetics are neither prepared nor diluted with pharmaceutical precision (Zawilska et al., 2021), adding an additional element of risk for overdose because of their high potencies relative to many prescription opioids and heroin. Analyses of counterfeit pills laced with fentanyl revealed more than a 10-fold variation in content, in one instance ranging between 0.6 and 6.9 mg/pill. Plasma fentanyl concentrations in overdose victims who had ingested these pills ranged from 7.9–162 ng/ml (Sutter et al., 2017), which is remarkable because the  $EC_{50}$  for respiratory depression is reported to be ~3.5 ng/ml (Moss & Carlo, 2019). Other studies have also reported a wide range of blood fentanyl concentrations in fatal overdoses, varying between 3 and



**Fig. 3.** Differences in signal transduction between synthetic (isotonitazene, fentanyl) and plant-derived (morphine, hydromorphone) opioids. HEK-293 T cells stably expressing the  $\mu$  opioid receptor (MOR) linked to either  $\beta$ -arrestin2 in the presence of coexpressed G protein-coupled receptor kinase 2 (MOR- $\beta$ -arr2; left panel) or “mini-Gi” (the GTPase domain of the  $G\alpha_i$  subunit) [MOR-mini-Gi, right panel] were used to evaluate the ability of opioids to recruit  $\beta$ -Arr2 and miniGi, respectively, using a luciferase-based reporter system as described (Vandeputte et al., 2021). Compounds (1 pM–100  $\mu$ M) were evaluated in five independent experiments with each concentrate run in duplicate. Values represent the X  $\pm$  SEM normalized to the maximum response of hydromorphone. Data are from Fig. 5a in Vandeputte et al. (2021), with permission.

383 ng/ml and 5–152 ng/ml, respectively (Martin, Woodall, & McLellan, 2006; Thompson et al., 2007). In a study of 20 survivors of non-fatal overdose, Krotulski et al. (2021) reported that 95% of the victims were exposed to fentanyl, with blood concentrations ranging from 0.1–19 ng/ml (mean 6.2 ng/ml).

Because every overdose is the product of a unique set of circumstances (Table 1), the “window of opportunity” to effect a successful rescue will vary among victims. Nonetheless, Fairbairn, Coffin, and Walley (2017) have suggested that a heroin overdose may not be lethal for at least 20–30 min, whilst an intravenous fentanyl overdose can produce life-threatening respiratory depression within 2 min. This suggestion is consistent with reports that respiratory depression was maximum at 5 min following intravenous administration of fentanyl (Harper, Hickey, Cromwell, & Linwood, 1976) and anecdotal reports of individuals witnessing a synthetic opioid overdose describing a manifestation of symptoms within seconds to minutes (Sommerville et al., 2017).

Adding to the potential risks associated with the misuse of synthetics, pharmacologically relevant doses of intravenous fentanyl and its analogs can produce rigidity in the chest wall and diaphragm as well as laryngospasm. This set of symptoms is collectively known as Wooden Chest Syndrome (WCS) (Torralva and Janowsky, 2019; Zibbell et al., 2019). While uncommon, this syndrome evolves rapidly (within 2 min after injection) and because it is life threatening, managed in the operating room with intravenous muscle relaxants such as succinylcholine and endotracheal intubation (Torralva and Janowsky, 2019). The mechanisms responsible for Wooden Chest Syndrome are not fully understood (Torralva and Janowsky, 2019), but are likely initiated by activation of  $\mu$  opioid receptors (Lalley, 2003) which modulate both cholinergic and adrenergic pathways regulating respiratory mechanics and airway patency (reviewed in Torralva and Janowsky, 2019). Although high doses of opiates (morphine and heroin) have been reported to produce abdominal rigidity, WCS appears linked to the rapid intravenous delivery of relatively high doses of fentanyl (and analogs) in the operating room. It is uncertain the extent to which WCS contributes to the mortality linked to overdose with synthetics but reports of difficulty performing cardiopulmonary resuscitation due to chest wall rigidity in overdoses involving synthetics (Baumann et al., 2018; Fairbairn et al., 2017) suggest that symptoms resembling WCS can complicate the management of synthetic opioid overdose. Although there is evidence WCS dissipates with naloxone administration (Zibbell et al., 2019; Torralva and Janowsky, 2019), given its rapid onset and life-threatening symptoms, it may not be adequately managed by doses of naloxone that reverse respiratory depression (Torralva and Janowsky, 2019).

*In toto*, both the physicochemical and pharmacological properties of synthetic opioids contribute to a shortened window of opportunity to intervene in an overdose compared to opiates. Nonetheless, regardless of the opioid(s) responsible for an overdose, if respiratory depression is the primary driver of morbidity and mortality, then reinitiating respiration and normalizing minute ventilation as quickly as possible reduces the likelihood of enduring hypoxic damage, potentially preventing a lethal overdose or minimizing the consequences associated with a nonfatal overdose.

#### 4.2. Naloxone: the prototypic rescue agent

A high affinity, competitive opioid antagonist, naloxone is listed as an “essential medicine” by the World Health Organization. For over five decades, naloxone has been the “gold standard” for the treatment of opioid overdose and is currently the only rescue agent approved by the U.S. Food and Drug Administration (FDA) (Skolnick, 2018b). Initially, naloxone use was largely confined to the ED; when administered intravenously in this setting, dose titration is employed to reverse the clinical signs of overdose whilst minimizing the potential for precipitating acute withdrawal in opioid-dependent individuals (Boyer, 2012). However, in the late 1990s the initial treatment of opioid overdose

began to shift from the ED to first responders (police, EMS, and the friends and family of victims) (Skolnick et al., 2018a). Many first responders, often either unable or unwilling to administer an injection, relied on improvised intranasal naloxone kits to rescue overdose victims. These kits, championed by the harm reduction community, contained 1–2 syringes prefilled with 2 ml of naloxone (either 0.4 or 1 mg/ml) and a mucosal atomizing device. The proper assembly and use of these improvised nasal delivery devices require training, and human factors analyses demonstrated a high error rate associated with assembly and use despite training (Edwards et al., 2015). While these improvised devices were extensively used and reported to be effective (Doe-Simkins, Walley, Epstein, & Moyer, 2009), plasma naloxone concentrations are substantially below (Krieter, Chiang, Gyaw, & McCann, 2019) those produced by FDA approved products intended for use by first responders: an auto-injector (0.4 and 2 mg) and a concentrated (4 mg/0.1 ml) intranasal formulation. Although these fixed dose products cannot be titrated to effect, human factors analyses indicate little or no training is needed for proper use (Edwards et al., 2015; Krieter et al., 2016). Sales of the naloxone auto-injector (Evzio® and generic equivalent) were halted in 2020 for commercial reasons (<https://www.drugs.com/availability/generic-evzio.html>). Intranasal naloxone (4 mg) (NARCAN® Nasal Spray) is widely used by first responders because of a rapid onset with peak plasma concentrations comparable to a 2 mg IM injection (Krieter et al., 2016; Krieter, Chiang, Gyaw, & McCann, 2019).

The effective dose of naloxone required to reverse an overdose is symptom driven and therefore empirical. However, in the face of an increased availability and misuse of illicit synthetics as well as their use as adulterants in other abused substances including heroin, cocaine, and benzodiazepines (Drug Enforcement Administration, 2021), converging lines of evidence indicate higher doses of naloxone may be needed to effect a successful rescue than are typically used by many first responders (e.g., 4 mg of nasal naloxone) (Sutter et al., 2017; Faul et al., 2017; Lynn and Galinkin, 2018; Moss and Carlo, 2019; Mahonski et al., 2019; Krieter, Gyaw, Chiang, Crystal, & Skolnick, 2019; Baumann et al., 2018; Moss et al., 2020; Pergolizzi, Dahan, LeQuang, & Raffa, 2021). Some authors (Li, Armenian, Mason, & Grock, 2018; Schumann, Erickson, Thompson, Zautcke, & Denton, 2008) have recommended parenteral naloxone doses of up to 12–15 mg if a synthetic opioid like fentanyl is suspected in an overdose. Consistent with these reports, the Health Advisory Network (a component of the CDC) recently issued a health advisory (Health Alert Network, 2020) stating: “...that multiple doses of naloxone may be needed for a single overdose event because of the potency of illicitly manufactured fentanyl and fentanyl analogs, and that multiple doses may be needed over time due to prolonged effects of opioids in some cases” (Health Alert Network, 2020). Preclinical data is consistent with the need for higher doses of naloxone to reverse a synthetic opioid overdose. Hill et al. (2020) reported mice required a 10-fold higher dose of naloxone (3 versus 0.3 mg/kg, i.p.) to reverse the respiratory depressant effects of fentanyl (0.15 mg/kg, i.p.) compared to morphine (10 mg/kg, i.p.) administered at equi-effective doses (Hill et al., 2020).

It is not surprising that overdose victims who required high doses of prehospital naloxone were more likely to be transferred to an intensive care unit (ICU) or step-down unit from the emergency department (Maloney et al., 2020). In a retrospective chart review of >513 patients who received prehospital naloxone, Maloney et al. (2020) reported individuals administered >2- < 4 mg of parenteral naloxone were 2.7-fold more likely to be admitted to either an intensive care or step-down unit compared to individuals who received  $\leq$  2 mg. The odds ratio for admission to either an intensive care or step-down unit increased to 3.7 and > 27, respectively, for victims receiving 4–6 mg and > 6 mg of prehospital naloxone. These data were collected at a suburban tertiary care center between 2014 and 2017, when synthetic opioid misuse was rising (Ahmad et al., 2021). While IN naloxone was most frequently used as a rescue agent in this study, victims rescued via the

IN route were excluded from the analysis because the bioavailability of naloxone administered with improvised IN kits is low and highly variable (Krieter, Chiang, Gyaw, & McCann, 2019), and an FDA approved IN naloxone product was not commercially available until mid-2016. Admission to an ICU or stepdown unit is indicative of an increased potential for overdose-related morbidities and adds substantially to treatment costs.

#### 4.3. Naloxone alternatives

The recent FDA approval of a higher dosage (8 mg) nasal naloxone product (<https://www.fda.gov/news-events/press-announcements/fda-approves-higher-dosage-naloxone-nasal-spray-treat-opioid-overdose>) offers an alternative to the widely used 4 mg product, but more innovative solutions are needed in an era when more than 80% of opioid overdose deaths have been linked to synthetics (Ahmad et al., 2021). Both pharmacological (e.g., AMPA receptor potentiators, serotonin receptor subtype selective agonists, calcium-activated potassium channel blockers) and physical (e.g., cyclodextrin scaffolds that bind fentanyl and related molecules, antibody-based strategies that prevent opioids from entering the central nervous system) approaches have been proposed to treat opioid overdose (Dahan et al., 2010; France et al., 2021; Roozenkrans et al., 2014; Skolnick, 2018b). However, the FDA approval pathway for a novel rescue agent, used either alone or in combination with naloxone, is uncertain, and presents a high and perhaps unacceptable degree of regulatory risk. An alternative approach with lower regulatory risk is developing a MOR antagonist which by dint of its pharmaceutical properties is a more effective rescue agent. An antagonist with higher affinity than naloxone combined with a formulation that results in rapid absorption (that is, a low  $T_{max}$ ) and/or higher concentration ( $C_{max}$ ) should produce more effective reversal of opioid-induced respiratory depression. This hypothesis assumes no substantive differences in the diffusion of these molecules between plasma and the effect compartment (i.e., the CNS) (Algera et al., 2019). Multiple MOR antagonists have been described with higher affinities than naloxone (Bidlack et al., 2018; Cassel, Daubert, & DeHaven, 2005; Kelly et al., 2015). Among these molecules, naltrexone, nalmefene, and most recently, samidorphan, have received regulatory approval, whilst GSK 1521498 has been in clinical development (Rabiner et al., 2011; Bidlack et al., 2018; Kahn et al., 2021), which substantially de-risks the single use of these molecules as rescue agents. In this context, nalmefene, currently approved in Europe for the treatment of alcohol use disorder, was approved as an injection by the FDA (1995) for reversal of opioid overdose, but withdrawn from the market for commercial reasons with no safety or efficacy concerns (<https://www.federalregister.gov/documents/2017/11/03/2017-23952/determination-that-revex-nalmefene-hydrochloride-injection-01-milligram-basemilliliter-and-10>).

The apparent affinity of naloxone at MOR has been examined under multiple experimental conditions, and the reported values are generally in the low (i.e.,  $\sim 1$ - $<10$ ) nM range. While differences in assay conditions (e.g., radioligand, buffer, receptor source) across studies can affect apparent affinity, a subset of studies have directly compared the affinities of multiple opioid antagonists providing better insight into the relative potencies of these molecules. For example, using radioligand binding techniques, Cassel et al. (2005) reported nalmefene and naltrexone were 4.9–5.4 and 3–6.3-fold more potent than naloxone, respectively depending upon assay conditions. These values were obtained under equilibrium binding conditions using two different radioligands and cell membranes prepared from cloned human MOR. When measured under non-equilibrium conditions, nalmefene and naltrexone were 3.6 and 5.1 more potent than naloxone, respectively (Cassel et al., 2005). These relative potency differences are consistent with radioligand binding studies using MOR from non-human primate brain, where nalmefene and naltrexone were reported to be 4.8 and 5.6-fold more potent than naloxone, respectively (Emmerson, Liu, Woods, & Medzihradsky, 1994). Bidlack et al. (2018) compared the

affinity of samidorphan to both naltrexone and naloxone under a variety of assay conditions in cell membranes expressing human MOR. Samidorphan was between 2 and 5-fold more potent than naltrexone and 10–20-fold more potent than naloxone, respectively. Potency differences among opioid antagonists have also been reported in functional assays. For example, Kelly et al. (2015) reported that GSK1521498 and nalmefene were both  $\sim 8.6$ -fold more potent than naloxone whilst naltrexone was  $\sim 2.9$ -fold more potent inhibiting Met-enkephalin stimulated [ $^{35}$ S]GTP $\gamma$ S binding to membranes prepared from cells overexpressing human MOR. These data are consistent with a study using “classical” organ bath preparations from guinea pig ileum and mouse vas deferens. Toll et al. (1998) reported that nalmefene and naltrexone were  $\sim 7.4$  and  $\sim 4.8$  fold more potent than naloxone, respectively, in both preparations.

The potential impact of using a more potent opioid antagonist can be modelled with drug plasma concentrations as a surrogate for target concentrations and the literature  $K_i$  values described above. The fraction of receptor bound by a ligand is defined in Eq. 1 (Bennett & Yamamura, 1985) as,

$$B = \frac{B_{max}L}{K_d + L} \quad (1)$$

and an analogous set of assumptions is used in imaging studies to estimate receptor occupancy (Nickolls et al., 2018). In this equation, B is the amount of ligand (drug) bound to receptor;  $B_{max}$  is the maximum amount of ligand bound to receptor; L is the ligand concentration;  $K_d$  is the equilibrium dissociation constant of the ligand, a measure of ligand affinity at the receptor.  $K_i$  is an analogous binding constant for the affinity of an unlabeled compound (Bennett & Yamamura, 1985) derived from competition studies using a radiolabeled ligand (e.g. Bidlack et al., 2018; Cassel et al., 2005).

Estimating receptor occupancy (Eq. 2) using plasma concentrations (5.3 ng/ml) of naloxone at  $T_{max}$  (30 min.) following a 4 mg IN dose (Krieter et al., 2016) as a measure of L and a  $K_i$  of 5.4 nM (Cassel et al., 2005), 0.75 of the maximum occupancy

$$B = \frac{B_{max} 5.3}{\left(5.4 \frac{\text{pmol}}{\text{ml}} \times 327 \frac{\text{pg}}{\text{pmol}}\right) + 5.3} = \frac{B_{max} 5.3}{1.76 + 5.3} = 0.75 B_{max} \quad (2)$$

(that is,  $\sim 75\%$  of the receptors) would be achieved by naloxone at  $T_{max}$ . In a PET study of MOR occupancy in healthy volunteers using [ $^{11}$ C] carfentanil as the imaging agent, Johansson et al. (2019) reported 85% receptor occupancy following a 4 mg dose of IN naloxone at  $T_{max}$ .

Using an opioid antagonist with a  $\sim 5$ -fold higher affinity (i.e., 1 nM) and assuming the same molecular weight and plasma concentration, receptor occupancy (Eq. 3) would be  $\sim 94\%$ :

$$B = \frac{B_{max} 5.3}{\left(1 \frac{\text{pmol}}{\text{ml}} \times 327 \frac{\text{pg}}{\text{pmol}}\right) + 5.3} = \frac{B_{max} 5.3}{0.327 + 5.3} = 0.94 B_{max} \quad (3)$$

Current FDA guidance for naloxone nasal spray calls for redosing the victim if no response is observed within 2–3 min. If there is no response (and assuming additional doses are available), additional doses are given every 2–3 min until emergency medical assistance arrives. Thus, examining receptor occupancy in the first minutes following antagonist administration is more relevant to a real-world rescue. Using study data from Krieter et al. (2016), plasma concentrations measured 5 min after dosing (1.48 ng/ml) with the 4 mg naloxone nasal spray results in  $\sim 45\%$  receptor occupancy (Eq. 4) whilst receptor occupancy with an opioid antagonist with  $\sim 5$ -fold higher affinity

$$B = \frac{B_{max} 1.48 \text{ ng/ml}}{\left(5.4 \frac{\text{pmol}}{\text{ml}} \times 327 \frac{\text{pg}}{\text{pmol}}\right) + 1.48 \text{ ng/ml}} = \frac{B_{max} 1.48}{1.76 + 1.48} = 0.45 B_{max} \quad (4)$$



$$B = \frac{B_{\max} 1.48 \text{ ng/ml}}{\left(1 \frac{\text{pmol}}{\text{ml}} \times 327 \frac{\text{pg}}{\text{pmol}}\right) + 1.48 \text{ ng/ml}} = \frac{B_{\max} 1.48}{0.327 + 1.48} = 0.82 B_{\max} \quad (5)$$

achieves ~82% receptor occupancy (Eq. 5). The nasal absorption of naloxone is linear within a range of 2–8 mg (Krieter et al., 2016). Based on the FDA guidance for naloxone dosing, it is instructive to examine the effect of doubling the plasma concentration of naloxone at 5 min: receptor occupancy rises to ~63%, well below that of the higher affinity agent. These estimates are based on plasma concentrations following intranasal dosing, but the higher receptor occupancy resulting from either a more potent opioid antagonist or a higher dose of naloxone (Moss et al., 2020) obtains independent of the route of administration. The difference in receptor occupancy following a higher affinity opioid antagonist is likely to be clinically meaningful because estimates of receptor occupancy are derived from the equation for a rectangular hyperbola, precluding full occupancy (i.e., values can approach, but never achieve 100% occupancy) (Bennett & Yamamura, 1985). Here, it is assumed that opioid antagonists act in a competitive fashion, and this simplistic modeling exercise does not consider the highly variable features (Table 1) attending an overdose. Nonetheless, this modeling exercise is consistent with the hypothesis that the use of a higher affinity competitive opioid antagonist favors a successful rescue, and a call by NIH leadership for the development of "...stronger, longer-acting formulations of antagonists" (Volkow & Collins, 2017) which has been endorsed by the President's Commission On Combatting Drug Addiction and The Opioid Crisis (Christie et al., 2017) in the face of increasing numbers of overdose deaths linked to synthetic opioids. A more potent opioid antagonist that can be used as a rescue agent goes beyond a theoretical. Thus, a parenteral formulation of nalmefene was FDA approved (1995) to treat opioid overdose, but withdrawn from the market in 2008 for commercial reasons, with no safety or efficacy concerns (<https://www.federalregister.gov/documents/2017/11/03/2017-23952/determination-that-revex-nalmefene-hydrochloride-injection-01-milligram-basemilliliter-and-10>). The need for more potent opioid antagonists is underscored by the recent filing of an Abbreviated New Drug Application (ANDA) for nalmefene HCl injection, and the FDA granting this product a priority review (<https://www.businesswire.com/news/home/20210610005182/en/FDA-Accepts-Filing-of-Abbreviated-New-Drug-Application-and-Grants-Priority-Review-for-Nalmefene-HCl-Injection-for-the-Treatment-of-Known-or-Suspected-Opioid-Overdose>). Further, multiple clinical studies have demonstrated the feasibility of developing intranasal formulations of both naltrexone (Krieter, Gyaw, Chiang, et al., 2019; Wermeling, 2013) and nalmefene (Krieter, Gyaw, Crystal, & Skolnick, 2019) which, like naloxone nasal spray, could be used by first responders with little or no training. The addition of dodecyl maltoside (DDM), which acts as a nasal absorption enhancer by transiently opening the tight junctions between epithelial cells (Maggio & Pillion, 2013), increased the speed of absorption of both intranasal naltrexone and nalmefene, reducing  $T_{\max}$  from 30 to 12 min (Fig. 4), and 120 to 15 min, respectively (Krieter, Gyaw, Chiang, et al., 2019; Krieter, Gyaw, Crystal, & Skolnick, 2019). The  $T_{\max}$  of both nalmefene and naltrexone in formulations containing DDM are consistent with a more rapid rate of absorption than naloxone (Fig. 4), with a  $T_{\max}$  of 30 min. (Krieter et al., 2016; [https://www.accessdata.fda.gov/drugsatfda\\_docs/label/2015/208411lbl.pdf](https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/208411lbl.pdf)). If receptor occupancies are calculated for naltrexone at 5 min based on plasma concentrations (Fig. 4) in the presence and absence of DDM (assuming an affinity of 0.86 nM at MOR; Cassel et al., 2005), there is an obvious advantage to using the higher affinity antagonist: 83.7% occupancy which is magnified to 97.5% occupancy in the presence of DDM. If this more rapid rate of absorption translates to a more rapid onset of action then this attribute, combined with a higher affinity at MOR could result in a more effective rescue agent.

The higher affinities of nalmefene and naltrexone compared at MOR to naloxone described *in vitro* are reflected as higher potencies *in vivo*.

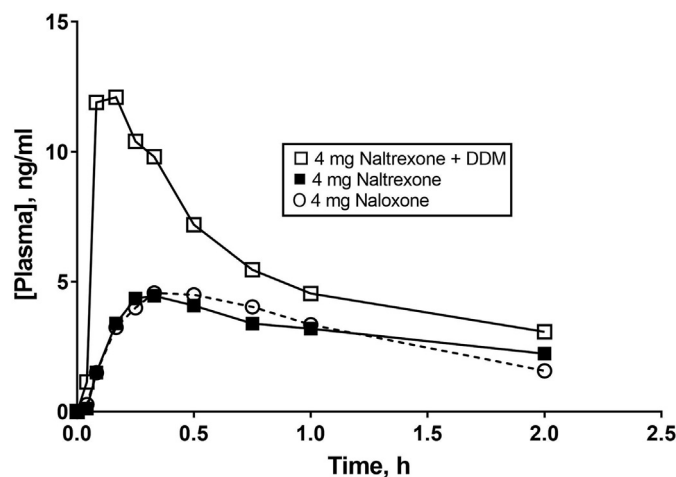


Fig. 4. Dodecyl maltoside (DDM) enhances the absorption of intranasal naltrexone. Naltrexone HCl (4 mg) was administered (100  $\mu$ l) intranasally to healthy volunteers in the presence (open squares) or absence (filled squares) of 0.25% (w/v) DDM. Values represent the mean plasma naltrexone concentrations (ng/ml) of 12–13 subjects. Included for comparison are plasma concentrations of naloxone following the intranasal administration (100  $\mu$ l) of naloxone HCl (4 mg) to healthy volunteers (open circles) which represent the mean plasma concentrations of 29 subjects. Methodological details and values for naltrexone and naloxone (partial data sets) are from Krieter, Gyaw, Chiang, et al. (2019) and Krieter et al. (2016).

For example, Yong et al. (2014) modelled an opioid overdose and subsequent rescue by administering rats an incapacitating intravenous bolus of carfentanil (10  $\mu$ g/kg) followed 5 min later by an intramuscular dose of either nalmefene (9.4–150  $\mu$ g/kg) or naloxone (150  $\mu$ g/kg) (Fig. 5). Nalmefene, at doses as low as 9.4  $\mu$ g/kg significantly reduced the duration of carfentanil-induced loss of righting reflex; at doses between 9.4 and 18.8  $\mu$ g/kg, the duration of loss of righting reflex was reduced to the same extent as 150  $\mu$ g/kg of naloxone indicating there could be an ~10-fold difference in potency between naloxone and nalmefene in this measure (Fig. 5). At a higher, respiratory depressant dose of carfentanil (20  $\mu$ g/kg), nalmefene (37.5–150  $\mu$ g/kg) produced a near complete-to-complete reversal within 10 min., normalizing the partial pressures of oxygen, carbon dioxide, and oxygen saturation in arterial blood to pre-carfentanil values. By comparison, naloxone (150  $\mu$ g/kg) produced a partial, albeit significant reversal of carfentanil-induced changes in blood gases. While interpretation of these data is limited by the use of a single dose of naloxone, nalmefene doses of 9.4–18.8  $\mu$ g appears to be equi-effective with naloxone (150  $\mu$ g/kg) in

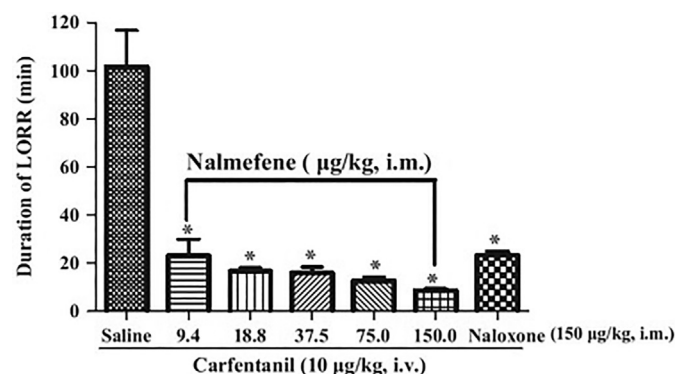


Fig. 5. Effect of nalmefene and naloxone on the duration of carfentanil-induced loss of righting reflex (LORR) in rats. Nalmefene or naloxone was injected (IM) 5 min after carfentanil administration (10  $\mu$ g/kg, i.v.). Bars represent  $\bar{X} \pm \text{SEM}$  of 5 rats. The righting reflex was deemed "lost" (Yong et al., 2014) if an animal did not right itself within 15 s of being placed on its back. The data is from Fig. 1A, Yong et al. (2014), with permission.

reversing carfentanil-induced changes in arterial blood gases measured 10 min. Post administration. In a subsequent study comparing the effects of intramuscular naloxone and naltrexone on the respiratory dynamics of mice exposed to aerosolized carfentanil, [Tuet et al. \(2019\)](#) reported that naloxone (1 and 5 mg/kg, i.m.) produced a marginal improvement in minute volume (whether given 15 min. Pre-carfentanil as a prophylactic measure or 15 min after conclusion of exposure as a rescue agent) in carfentanil exposed mice, exhibiting only modest dose dependent effects. In contrast, 1 and 5 mg/kg of naltrexone returned minute ventilation to baseline and reduced the duration of respiratory depression compared to naloxone. The authors concluded that "...naltrexone performed more favorably than naloxone", although no clear inference can be drawn about the relative potency difference between these two opioid antagonists.

Clinical evidence that the potency differences observed in these pre-clinical models translate to a therapeutic advantage is less compelling. There is one double blind study ([Kaplan et al., 1999](#)) comparing the effects of nalmefene and naloxone in patients admitted to EDs with a suspected narcotic overdose. In this multicenter study, 156 patients were randomized to receive either naloxone (2 mg) or nalmefene (1–2 mg) intravenously every 5 min as needed, with a maximum of 4 doses. Most patients received only one dose of study drug, and in those individuals with a confirmed opioid overdose (~43%) both naloxone and nalmefene rapidly reversed respiratory depression. This study was done in the 1990s, a period when prescription opioids and heroin were responsible for most opioid overdoses, so it is perhaps not surprising there were no readily quantifiable differences in effectiveness between intravenous naloxone and nalmefene in an ED setting, and that the great majority of patients responded to a single intravenous dose of either reversal agent. However, two small studies provide some indication that the potency differences between nalmefene and naloxone described in preclinical studies have clinical relevance. [Glass, Jhaveri, and Smith \(1994\)](#) compared the ability of intravenous bolus doses (1, 2, 4, or 8 µg/kg) of nalmefene and naloxone to reverse the respiratory depressant effects of a fentanyl infusion in healthy volunteers breathing a gas mixture enriched in CO<sub>2</sub>. There was a significant effect of dose, but no statistically significant difference was found between drugs because of the high variability among subjects. Nonetheless, visual inspection of these data indicates the doses of nalmefene and naloxone that produce a 50% recovery of slope (a measure of reversal of the respiratory depressant effects of fentanyl) was ~1 and ~3 µg/kg, respectively. Consistent with these data, in a crossover study examining morphine-induced respiratory depression under conditions of elevated CO<sub>2</sub> in six healthy volunteers, [Konieczko, Jones, Barrowcliffe, Jordan, and Altman \(1988\)](#) reported an intravenous nalmefene bolus of 0.4 mg produced a reversal of minute ventilation that was numerically superior to (but not statistically significantly different from) 1.6 mg of naloxone. The reversal produced by the 0.4 mg dose of nalmefene was also higher ( $p = 0.08$ ) than produced by a 0.4 mg dose of naloxone. The apparent differences in potency between nalmefene and naloxone were noted between 0 and 1.5 h after drug administration; the differences between nalmefene and naloxone were more apparent and reached statistical significance when measured at 1.5–4.5 and 4.5–6 h post-dosing. The apparent change in potency over time is not unexpected because of the differences in plasma half-lives of naloxone ( $t_{1/2}$  1–2 h) [[Ryan & Dunne, 2018](#)] and nalmefene ( $t_{1/2}$  8–10) [[Dixon et al., 1986](#)].

Multiple high affinity opioid antagonists, including GSK 1521498 (22.7 h) [[Ziauddeen et al., 2013](#)], naltrexone (6.4 h) [[Krieter, Gyaw, Crystal, & Skolnick, 2019](#)], nalmefene (8–10 h) [[Dixon et al., 1986](#)], and samidorphan (7–9 h) [[Turncliff, DiPetrillo, Silverman, & Ehrlich, 2015](#)] have longer plasma half-lives than naloxone ( $t_{1/2}$  1–2 h) [[Ryan & Dunne, 2018](#)]. Longer half-lives reduce the likelihood that symptoms of overdose (including respiratory depression) will reoccur if longer acting opioids are involved and concentrations of naloxone fall below

therapeutically effective levels. This phenomenon, known as re-narcotization, complicates the management of overdose, with the potential for additional naloxone administered as either a bolus or infusion ([Wang, Sternbach, & Varon, 1998](#)). In a retrospective study conducted over an 8-year period, evidence of a re-narcotization event following an initial response to naloxone was observed in 31% of adult patients in an emergency department setting ([Watson, Steele, Muelleman, & Rush, 1998](#)). In this study, re-narcotization was reported to be more common with long acting opioids, and was not associated with either the route of administration or the presence of other CNS depressants (e.g. ethanol). A re-narcotization event is especially dangerous if an overdose victim, administered naloxone and temporarily alert, refuses additional treatment ([Kaplan & Marx, 1993](#)). While the long half-lives of both methadone ( $t_{1/2}$  8–59 h) (<https://www.fda.gov/media/76020/download>) and buprenorphine ( $t_{1/2} > 30$  h) ([https://www.accessdata.fda.gov/drugsatfda\\_docs/label/2018/020732s0181bl.pdf](https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/020732s0181bl.pdf)), commonly used in the treatment of OUDs can complicate the management of overdose, even more problematic is that neither the pharmacokinetic nor pharmacodynamic properties of many synthetic opioids available on the "gray market" have been characterized ([Armenian, Vo, Barr-Walker, & Lynch, 2018](#); [Suzuki & El-Haddad, 2017](#)). As described earlier, the high lipophilicity of synthetics results in an initial rapid fall in plasma levels followed by a much slower decline ([Ahonen et al., 2000](#); [Armenian et al., 2018](#); [Suzuki & El-Haddad, 2017](#)). This initial fall can lead to a clinical impression that synthetics like fentanyl have a short half-life ([Mars et al., 2019](#)), but a complex pharmacokinetic profile with a plasma  $t_{1/2}$  estimated in multiple clinical studies at 7–8 h ([Ahonen et al., 2000](#); [Holley & Van Steenis, 1988](#); [Scott & Stanski, 1987](#)) increases the probability for a re-narcotization event in victims rescued with naloxone.

#### 4.4. Potential drawbacks using high affinity, long-acting opioid antagonists as rescue agents

Together with the potential advantages of using high affinity, long duration opioid antagonists as rescue agents comes a potential for severe and sustained withdrawal in victims who are opioid dependent. Nonetheless, in the double-blind study ([Kaplan et al., 1999](#)) comparing the effectiveness of intravenous nalmefene (1 or 2 mg) and naloxone (2 mg) in overdose, there were no dramatic differences in either the incidence or duration of withdrawal events among treatment groups. Adverse events in confirmed opioid positive patients were noted in all three treatment arms: 3/24 (12.5%) in the 2 mg naloxone group, 3/30 (10%) in the 1 mg nalmefene group, and 6/23 (26.1%) in the 2 mg nalmefene group ( $P > 0.27$ ). The relevance of this 25-year-old ED-based study to the present, when synthetic opioids are linked to most overdoses ([Ahmad et al., 2021](#); [Pardo et al., 2019](#)) and the initial management of overdose is most often in the hands of first responders, is unknown. Nonetheless, in a survey of organizations using nasal naloxone (4 mg) in a community setting, the incidence of withdrawal related events noted as "withdrawal" (14.3%; 28/196), "nausea", "vomiting", or "gagging-retching" (10.2%) and "irritability" or "anger" (8.7%) ([Avetian et al., 2018](#)) did not differ remarkably from the incidence of adverse events reported by [Kaplan et al. \(1999\)](#) following a 2 mg IV dose of naloxone, which would result in significantly higher plasma concentrations delivered far more rapidly than via the intranasal route ([McDonald et al., 2018](#)). In this community-based setting ([Avetian et al., 2018](#)), heroin was identified as the opioid involved in the overdose >95% of the time, and most reversals (97.6%) required a single dose of nasal naloxone. However, in an era when higher naloxone doses may be required for a successful rescue, there is the potential to produce a more severe and/or sustained withdrawal (but see [Lynn and Galinkin, 2018](#)) which could lead to subsequent drug use post-rescue. Despite such concerns, [Neale and Strang \(2015\)](#) recently reported that in a study of 47

overdose events in New York City, withdrawal symptoms were reported in 36% victims rescued with naloxone, but neither the presence of withdrawal symptoms nor feelings of anger in rescue victims were associated with drug use post reversal.

Multiple authors have raised concerns that “over-antagonism” can result in iatrogenic harm (Neale & Strang, 2015; Lynn and Galinkin, 2018; Farkas et al., 2020), especially in an era when many overdose victims are initially treated with fixed dose products rather than titration to effect by IV dosing. Perhaps most concerning are reports of pulmonary complications, including pulmonary edema, in overdose victims following naloxone administration (Clarke et al., 2005; Lynn and Galinkin 2018). In a recent retrospective study of over 1800 patients receiving out of hospital naloxone, Farkas et al. (2020) reported that overdose victims receiving high doses (>4.4 mg) were significantly more likely (odds ratio 2.14) to have pulmonary complications (e.g., pulmonary edema, aspiration pneumonia, aspiration pneumonitis). In this cohort, pulmonary edema was noted in 1.1% of patients. These authors were unable to conclude this was a causal relationship, and state: “It stands to reason that pretreatment morbidity can have a correlational effect with the decision to administer additional naloxone to patients, and if such an effect were strong enough, it could even mask positive effects of high-dose naloxone administration....” (Farkas et al., 2020). This interpretation is consistent with other authorities (Clarke et al., 2005; Lynn and Galinkin, 2018), and summarized in Boyer’s (2012) review on the management of opioid overdose: “Naloxone has been mistakenly implicated as a cause of pulmonary edema. However, pulmonary edema is present in nearly all fatal cases of opioid overdose, including those that occurred before the development of naloxone. Moreover, studies have shown that pulmonary edema does not develop in patients who receive large doses of naloxone by means of continuous infusion. Finally, auscultatory signs of pulmonary edema, which are often obscure in patients with apnea, become apparent only after naloxone restores ventilation” (Boyer, 2012). Nonetheless, the range of withdrawal symptoms (including agitation, piloerection, nausea and vomiting, diarrhea, sweating, muscle cramps, hypertension, and tachycardia) which can be precipitated by administration of an opioid antagonist, while clearly unpleasant, are not life threatening and can be medically managed (Boyer, 2012). In an era when high potency synthetics are involved in most opioid overdoses (Ahmad et al., 2021; Pardo et al., 2019) and initial treatment frequently falls to first responders, the rapid delivery of high affinity antagonists increases the probability of a successful rescue by dint of a more rapid re-initiation of normal respiration, reducing the potential for enduring hypoxic damage. This principle obtains regardless of where the victim lies on the continuum of events associated with an opioid overdose.

#### 4.5. Conclusions

Given the likelihood of continued misuse of fentanyl and fentanyl analogs, the emergence (Thompson, 2020; Ujvary et al., 2021) of a structurally distinct class of illicit synthetic benzimidazoles which share many of the same characteristics that make fentanyl misuse so dangerous, and the concerns about weaponization of ultra-high affinity synthetics like carfentanyl (France et al., 2021; Shafer, 2019; Wax, Becker, & Curry, 2003), the development of more effective overdose reversal agents is one component of a strategy to reduce the impact of opioid misuse, and must be viewed as a public health priority (Christie et al., 2017; Volkow & Collins, 2017).

#### Declaration of Competing Interest

Phil Skolnick is an employee and stockholder of Opiant Pharmaceuticals, Inc. (<https://www.opiant.com>), a company developing medicines for the treatment of addictions and drug overdose. Opiant Pharmaceuticals is currently developing nasal nalmeferene for the treatment of opioid overdose.

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