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Quantifying enhanced risk from alcohol and other factors in polysubstance-related deaths

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ABSTRACT

Background: To quantify how alcohol, polysubstance use and other factors influence opioid concentrations in drug-related deaths in West Virginia (WV), United States.
Methods: Multiple linear regression models were employed to identify relationships among alcohol, other factors, and the concentrations of four commonly identified opioids (fentanyl, hydrocodone, oxycodone, methadone), accounting for demographic, toxicological and comorbid characteristics in WV drug-related deaths from 2005 to 2018.
Results: Alcohol concentrations of 0.08% or above were associated with significant reductions in blood concentrations of fentanyl (27.5%), hydrocodone (30.5%) and methadone (32.4%). Significantly lower predicted concentrations of all opioids studied were associated with multiple opioid vs. single opioid presence, with predicted concentration reductions ranging from 13.7% for fentanyl to 65–66% for hydrocodone and oxycodone. Benzodiazepine presence was associated with small, non-statistically significant changes in opioid concentrations, while stimulant presence was associated with statistically significant reductions in hydrocodone and oxycodone concentrations.
Conclusions: Co-ingestion of alcohol, multiple opioids or stimulants were associated with significantly decreased predicted concentrations of commonly identified opioids in drug deaths. Further evidence is provided for enhanced risks from polysubstance use with opioids, which has important public health implications.

1. Introduction

Unintentional drug-related deaths are an ongoing public health problem [1,2]. In 2017, West Virginia (WV) had the highest per capita drug overdose mortality rate (57.8 per 100,000) in the United States (U.S.) [1]. Similar to the rest of the nation, most deaths involved opioid analgesics, particularly synthetic opioids such as fentanyl, usually in combination with other opioids, benzodiazepines, stimulants, or antidepressants [2–4]. Alcohol (ethanol) was a co-intoxicant in 18.5% of opioid-related and 21.4% of benzodiazepine-related U.S. deaths in 2010 and in 11% of synthetic opioid-related deaths in 2016 [4,5]. Thus, alcohol can be an important and often underappreciated contributing factor in drug-related deaths.

Opioid-related deaths are primarily due to overdose and the resultant respiratory depressant effects. Alcohol intoxication also produces central nervous system (CNS) and respiratory depression [5,6], and as a co-intoxicant can produce additive or synergistic effects with other CNS depressants, reverse tolerance to opioid-induced respiratory depression, or alter the pharmacokinetic profile of various opioids [7–10]. Additive or synergistic alcohol co-intoxicant effects might also result in increased toxicity from lower concentrations of opioids, benzodiazepines or cocaine compared to single ingestions [11,12]. Benzodiazepines primarily depress respiration when combined with opioids in drug overdoses, but whether this combination results in pharmacokinetic or other interactions is unclear [13]. Previous research has found an inverse association between the number of co-intoxicants in drug-related deaths and the alcohol concentrations present. Jones et al. reported

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mean alcohol concentrations in postmortem femoral blood to be lower as the number of co-intoxicants increased: 306 mg% (alcohol alone), 215 mg% (one co-intoxicant), and 178 mg% (two co-intoxicants) [14].

Only a limited number of studies have examined the association of alcohol with the concentrations of other co-intoxicant drugs present in drug-related deaths, which can be an indirect measure of enhanced toxicity potential with these combinations. Alcohol, in concentrations above or below the legal U.S. limit for intoxication (0.08%), was associated in one multistate study with lower concentrations of hydrocodone, methadone, and oxycodone, but not fentanyl, in single opioid deaths involving these substances [15]. Alcohol was also found to have a greater association with lower methadone and oxycodone concentrations than benzodiazepines (alprazolam and diazepam). However, this study excluded cases involving multiple opioid ingestion and only included alprazolam and diazepam but no other benzodiazepines. Another study found lower median amitriptyline and propoxyphene concentrations when alcohol was a co-intoxicant in these deaths; thus, it was cautioned that additive or synergistic effects might occur among various drug/alcohol combinations [16]. Cone et al. reported that oxycodone levels were generally lower in the presence of benzodiazepines or alcohol in drug overdose deaths, although the presence of other drugs in the analyses makes this interpretation less clear [17].

With greater fentanyl, heroin, cocaine, and methamphetamine involvement in substance-related deaths in the United States [3], there is a need to better understand the associations among alcohol and other commonly identified substances, decedent characteristics (e.g., sex, age, comorbid cardiac or pulmonary disease), and postmortem opioid concentrations. This study sought to characterize and quantify the associations of alcohol, various co-intoxicants, and decedent characteristics with opioid concentrations in a large number of opioid-related deaths. It was hypothesized that when alcohol or other respiratory depressants are combined with opioids, toxicity would occur at lower opioid concentrations due to enhanced respiratory depression; underlying cardiovascular or pulmonary comorbidities could also predispose individuals to fatal outcomes at lower opioid concentrations.

2. Materials and methods

The WV Office of the Chief Medical Examiner (OCME) uses a centralized medical examiner system in which death scene investigations, autopsies, comprehensive drug screening, and quantitated toxicology testing are performed in suspected drug deaths. WV routinely identifies on the death certificate the specific substances involved in a death, including alcohol. A forensic drug database (FDD) was created in 2005 to compile data from all WV drug-related deaths, with ongoing data entry. Each FDD case includes decedent demographics (age, sex, zip code, location of death), body condition, body mass index (BMI), cause and manner of death, drugs identified as a direct cause of or contributor to death, whether the decedent had a prescription (within the previous 30 days) for any controlled substances identified, medical history (when available), key autopsy findings, and toxicological analyses.

All drug-related deaths in the FDD from January 2005 to August 2018 were identified. Drug-related deaths were defined as deaths for which the OCME determined that at least one drug or alcohol was a direct cause of or a contributor to death and the manner of death was ruled unintentional. The study sample included all decedents in which at least one of the following four commonly encountered opioids, fentanyl, hydrocodone, oxycodone, and methadone, was identified as a cause of or contributor to death. Heroin, although frequently involved in deaths, is usually not present in postmortem blood samples as the parent drug due to rapid metabolism to 6-monoacetylmorphine, so its blood concentrations can generally not be determined. Only peripheral blood concentrations (femoral or subclavian) were included in our toxicological analyses. Reasons for exclusion from the study sample were: other than unintentional death (i.e., suicide, undetermined, homicide), moderate or severe body decomposition, missing BMI data, and lack of femoral or subclavian blood concentration data for the opioids studied.

Drug screening is routinely performed on all deaths investigated by the WV OCME, with confirmative toxicology tests conducted for most positive screens, including therapeutic and nonprescription drugs. Blood and/or tissue samples are screened for volatile compounds using gas chromatography with flame ionization detection and for drugs of abuse using automated enzyme immunoassays. This latter test includes: amphetamines, barbiturates, benzodiazepines, buprenorphine, cocaine, fentanyl/fentanyl analogs, methadone, opiates (morphine, codeine, hydrocodone, hydromorphone, oxycodone, oxymorphone) and marijuana. In general, alcohol was considered to have been ingested (i.e., not an artifact secondary to decomposition) if it was listed on the death certificate as a cause of or contributor to death, if only mild or no body decomposition was present, and if concentrations were at least 0.02%. Vitreous fluid or urine were used to confirm the presence of alcohol. Table 1 shows the limits of quantitation (LOQ) and analytical methods used for the compounds included in this study.

The primary outcome measures were the concentrations of the four opioids (fentanyl, hydrocodone, oxycodone, and methadone) studied. Demographic, toxicological and comorbid characteristics of the overall sample were characterized using descriptive statistics. Multiple linear regression models were used to examine relationships between the opioid concentrations and several decedent characteristics and comorbidities. Predictors included sex, age and BMI, as well as presence or absence of each of the following: 1) opioids (defined as multiple opioids vs. single opioid present), 2) benzodiazepines, 3) stimulants, 4) alcohol (defined as absent, present at $< 0.08\%$ or present at $\geq 0.08\%$), 5) tricyclic antidepressants (TCAs), 6) selective-serotonin reuptake inhibitors (SSRIs), 7) diphenhydramine (DPH), 8) cardiovascular disease, 9) pulmonary disease, and 10) history of substance abuse. Cardiovascular disease (i.e., cardiomyopathy, cardiomegaly, hypertrophy, hypertensive cardiovascular disease, ischemic heart disease, atherosclerotic coronary artery disease, cardiac disease, heart failure, myocardial infarction) and pulmonary disease (asthma, sleep apnea, chronic obstructive pulmonary disease, and pneumoconiosis) were identified through autopsy reports, medical records (when available) or other applicable information in decedent records. A history of substance abuse included drug, alcohol, and/or other substance abuse and was determined from medical records and/or other information in decedents' files (e.g., family/friend reports, death scene/police investigations).

Due to the skewed nature of the concentrations, log-transformed opioid concentrations were modeled to satisfy the linear regression assumptions. For each opioid of interest, separate multiple linear regression models were applied to examine associations between log-opioid concentrations and each of the following factors, while adjusting for the others: age, sex, BMI, alcohol concentration, and the presence of the following: another opioid(s), benzodiazepines, stimulants, TCAs, SSRIs, diphenhydramine, cardiovascular disease, pulmonary disease and a history of substance abuse. The analytic approach of multiple linear regression used in this study enables estimation of alcohol effects on log-opioid concentrations while accounting for the effects of other important factors, such as multiple opioid and stimulant

Table 1
Limits of Quantitation and Analytical Methods Used for the Compounds.

Compound	LOQ	Analytical Method
Ethanol	0.02%	GC/FID
6-Monoacetylmorphine	0.01 mg/L	LC/MS/MS
Codeine	0.01 mg/L	LC/MS/MS
Hydrocodone	0.01 mg/L	LC/MS/MS
Hydromorphone	0.01 mg/L	LC/MS/MS
Morphine	0.01 mg/L	LC/MS/MS
Oxycodone	0.01 mg/L	LC/MS/MS
Oxymorphone	0.01 mg/L	LC/MS/MS
Acetyl Fentanyl	0.5 ng/mL	LC/MS/MS
Fentanyl	0.5 ng/mL	LC/MS/MS
Norfentanyl	0.5 ng/mL	LC/MS/MS
Buprenorphine	1.0 ng/mL	LC/MS/MS
Norbuprenorphine	1.0 ng/mL	LC/MS/MS
Fentanyl Analogs	25 pg/mL	LC/MS/MS
Meperidine	0.02 mg/L	GC/MS
Methadone	0.02 mg/L	GC/MS
Methadone Metabolite (EDDP)	0.02 mg/L	GC/MS
Propoxyphene	0.02 mg/L	GC/MS
Tramadol	0.02 mg/L	GC/MS
7-Aminoclonazepam	0.01 mg/L	LC/MS/MS
Alprazolam	0.01 mg/L	LC/MS/MS
Chlordiazepoxide	0.01 mg/L	LC/MS/MS
Clonazepam	0.01 mg/L	LC/MS/MS
Diazepam	0.01 mg/L	LC/MS/MS
Lorazepam	0.01 mg/L	LC/MS/MS
Nordiazepam	0.01 mg/L	LC/MS/MS
Oxazepam	0.01 mg/L	LC/MS/MS
Temazepam	0.01 mg/L	LC/MS/MS
Cocaine	0.01 mg/L	LC/MS/MS
Benzoylecgonine	0.01 mg/L	LC/MS/MS
Ecgonine Methyl Ester	0.01 mg/L	LC/MS/MS
Amphetamine	0.01 mg/L	LC/MS/MS
Methamphetamine	0.01 mg/L	LC/MS/MS
Diphenhydramine	0.02 mg/L	GC/MS
Amitriptyline	0.02 mg/L	GC/MS
Amoxapine	0.02 mg/L	GC/MS
Clomipramine	0.02 mg/L	GC/MS
Desipramine	0.02 mg/L	GC/MS
Doxepin	0.02 mg/L	GC/MS
Fluvoxamine	0.02 mg/L	GC/MS
Imipramine	0.02 mg/L	GC/MS
Nortriptyline	0.02 mg/L	GC/MS
Citalopram	0.02 mg/L	GC/MS
Fluoxetine	0.02 mg/L	GC/MS
Paroxetine	0.02 mg/L	GC/MS
Sertraline	0.02 mg/L	GC/MS

LOQ: limits of quantitation.

presence and comorbidities. The percentage difference on the original concentration scale for each factor was calculated using the formula: $(e^{\beta}-1)\times 100\%$ (where β is the linear regression coefficient associated with that factor) to present the findings in a clinically relevant manner. Descriptive and regression analyses were performed using SAS 9.4 (SAS Institute, Cary, NC). Findings were considered statistically significant at $p<0.05$.

3. Results

A total of 9223 drug-related deaths were recorded in the FDD from January 2005 to August 2018 (Fig. 1). A total of 3922 deaths were included in the study sample for final analyses; 330 decedents (3.6%) were excluded for significant body decomposition and 1095 deaths (12.3%) were not unintentional. Among the remaining 7798 deaths, 4598 involved one of the four study opioids. Twelve additional cases had missing BMI data, and 664 lacked femoral or subclavian concentrations of the study opioids, resulting in a total study sample of 3922 deaths. Oxycodone was involved in the largest number of cases, followed by fentanyl, hydrocodone, and methadone.

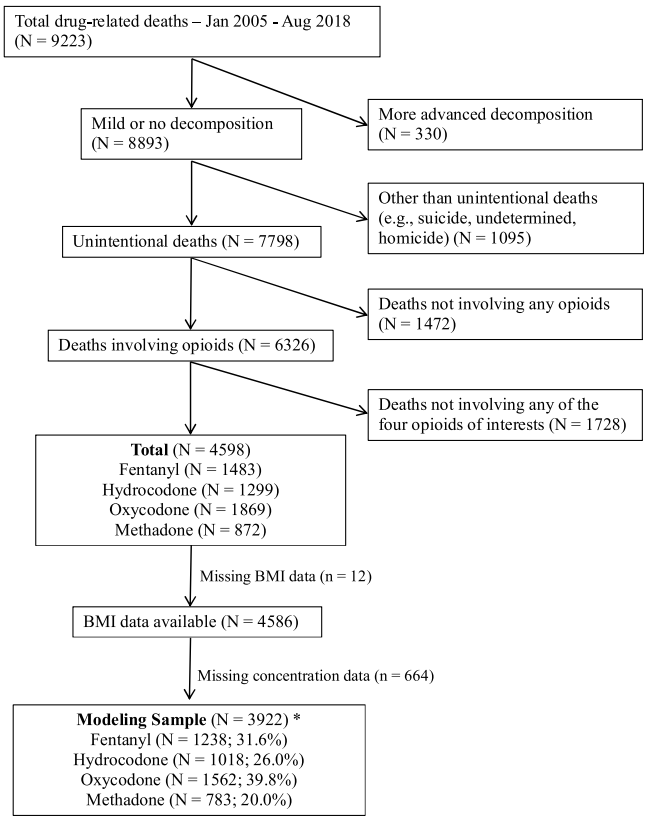

Fig. 1. Study Sample Case Identification among West Virginia Drug-related Deaths, 2005-2018.
* Sample sizes are not mutually exclusive due to possible multiple opioid presence.

The characteristics of the study opioid-related deaths are shown in Table 2. About 67% were male, with a mean decedent age of 40.8 years (range = 38–43 years across opioids). The mean BMI overall (29.2) in the deaths was in the upper overweight range and fairly consistent across opioids. Alcohol was present in about 10–18% of deaths involving the opioids studied, generally in concentrations of 0.08% or greater. Three of the four opioids studied, with the exception of methadone, were more likely to have other opioids present. A cross-tabulation of the number (%) of deaths involving the opioids studied is shown in Table 3. For example, of the 1238 cases involving fentanyl, hydrocodone was also identified in 9.7%, oxycodone in 8.4%, and methadone in 4.7% of those cases. Benzodiazepines were present in over half of all opioid-related deaths, ranging from roughly 37% of fentanyl deaths to 69% of hydrocodone deaths. Antidepressants (SSRIs, TCAs) and diphenhydramine were present in from 2 to 9% of the opioid-related deaths. Decedents with a history of drug and/or alcohol abuse varied substantially among the opioids studied, from 47% with methadone to 80% with fentanyl. Table 4 reports the estimated factor effects of the various predictors on the log-transformed opioid concentrations (log-μg/mL) in the regression models, with the alcohol effects shown in Table 5. Table 6 presents these effects as average percent changes in predicted drug concentrations using the original concentration scale of μg/mL (statistically significant findings shown in bold). Every 10-year increase in age was associated with relatively small (7.6%) but significant increases in oxycodone concentrations. Thus, an age change from 30 years to 50 years would be associated with about a 15% increase in predicted oxycodone concentrations. A change in BMI of 5 units was associated with a statistically significant decrease only in fentanyl concentrations (9%). Male sex was associated with statistically significant reductions of 16–17% in

Table 2
Characteristics of Study Opioid Deaths (N = 3922).

	Total	Fentanyl	Hydrocodone	Oxycodone	Methadone
Total number, N	3922	1238	1018	1562	783
Age, mean (SD)	40.8 (11.2)	38.9 (10.8)	43.3 (10.9)	42.5 (10.9)	38.0 (11.1)
BMI, mean (SD)	29.2 (7.8)	28.4 (7.1)	29.8 (7.9)	30.0 (8.2)	28.6 (7.3)
Male, N(%)	2643 (67.4)	870 (70.3)	654 (64.2)	1033 (66.1)	525 (67.0)
Substances present, mean (SD)	3.2 (1.4)	3.0 (1.4)	3.8 (1.5)	3.4 (1.5)	2.9 (1.4)
Ethanol					
Absent, N (%)	3323 (84.7)	1054 (85.1)	832 (81.7)	1326 (84.9)	704 (89.9)
< 0.08%, N (%)	201 (5.1)	73 (5.9)	45 (4.4)	86 (5.5)	29 (3.7)
> = 0.08%, N (%)	398 (10.1)	111 (9.0)	141 (13.9)	150 (9.6)	50 (6.4)
Opioids					
Single opioid present, N (%)	2004 (51.1)	467 (37.7)	332 (32.6)	715 (45.8)	490 (62.6)
Multiple opioids[a] present, N (%)	1918 (48.9)	771 (62.3)	686 (67.4)	847 (54.2)	293 (37.4)
Any benzodiazepine[b] present, N (%)	2204 (56.2)	455 (36.8)	705 (69.3)	1058 (67.7)	421 (53.8)
Any stimulant[c] present, N (%)	737 (18.8)	369 (29.8)	143 (14.0)	219 (14.0)	122 (15.6)
TCA present[d], N (%)	158 (4.0)	18 (1.5)	42 (4.1)	83 (5.3)	39 (5.0)
SSRI present[e], N (%)	260 (6.6)	43 (3.5)	81 (8.0)	122 (7.8)	72 (9.2)
DPH present, N (%)	189 (4.8)	27 (2.2)	57 (5.6)	76 (4.9)	66 (8.4)
Cardiovascular disease[f], N (%)	1704 (43.4)	439 (35.5)	489 (48.0)	791 (50.6)	295 (37.7)
Pulmonary disease[g], N (%)	831 (21.2)	193 (15.6)	274 (26.9)	369 (23.6)	149 (19.0)
History of substance abuse[h], N (%)	2376 (60.6)	994 (80.3)	546 (53.6)	863 (55.2)	368 (47.0)

TCA = tricyclic antidepressant, SSRI = selective serotonin receptor inhibitor, DPH = diphenhydramine, BMI = body mass index.
[a] Includes fentanyl and fentanyl analogs, hydrocodone, oxycodone, methadone, morphine, buprenorphine, codeine, heroin, hydromorphone, diphenoxylate, propoxyphene, meperidine, tramadol, oxymorphone.
[b] Includes alprazolam, clonazepam, chlordiazepoxide, diazepam, lorazepam, oxazepam, temazepam.
[c] Includes amphetamine, methamphetamine and cocaine.
[d] Includes amitriptyline, clomipramine, doxepin, imipramine, trimipramine, amoxapine, desipramine, nortriptyline, protriptyline.
[e] Includes citalopram, escitalopram, fluoxetine, paroxetine, sertraline, vilazodone, fluvoxamine.
[f] Includes cardiomyopathy, cardiomegaly, hypertrophy, hypertensive cardiovascular disease, ischemic heart disease, atherosclerotic coronary artery disease, cardiac disease, heart failure, myocardial infarction.
[g] Includes asthma, sleep apnea, chronic obstructive pulmonary disease, pneumoconiosis.
[h] Includes drug, alcohol or other substance.

Table 3
Cross-Tabulation of the Number (%) of Deaths Involving Opioid Studied.

	Fentanyl	Hydrocodone	Oxycodone	Methadone
Fentanyl	1238	99 (9.7)	131 (8.4)	37 (4.7)
Hydrocodone	99 (8.0)	1018	297 (19.0)	56 (7.2)
Oxycodone	131 (10.6)	297 (29.2)	1562	95 (12.1)
Methadone	37 (3.0)	56 (5.5)	95 (6.1)	783

Note: Percentages calculated by column.

methadone and fentanyl concentrations, with no significant associations found with the other concentrations.

Reductions in the predicted concentrations of all the opioids were associated with the presence of alcohol, with statistically significant decreases observed for fentanyl, hydrocodone, and methadone but not oxycodone. Both high and low alcohol concentrations (< 0.08% or $\geq$ 0.08%) were associated with significant reductions of about one third in hydrocodone concentrations, with higher alcohol concentrations ($\geq$ 0.08%) associated

Table 4
Adjusted Estimated Effects on Log-Opioid Concentration From Multiple Regression Models (log-μg/mL).

Factors	Coefficients (Standard error)			
	Fentanyl (N = 1238)	Hydrocodone (N = 1018)	Oxycodone (N = 1562)	Methadone (N = 783)
Age	−0.001 (0.003)	0.006 (0.003)	**0.007 (0.003)[a]**	0.003 (0.003)
BMI	**−0.019 (0.004)**[a]	−0.006 (0.004)	−0.003 (0.004)	0.006 (0.004)
Male	**−0.183 (0.070)**[a]	0.073 (0.070)	−0.027 (0.063)	**−0.174 (0.071)**[a]
Ethanol concentration main effect	**p = 0.01**	**p < 0.01**	p = 0.09	**p < 0.01**
Ethanol concentration <0.08% vs. Absent	−0.192 (0.133)	**−0.455 (0.160)**[a]	−0.217 (0.127)	−0.284 (0.170)
Ethanol concentration ≥0.08% vs. Absent	**−0.322 (0.110)**[a]	**−0.363 (0.098)**[a]	−0.149 (0.100)	**−0.392 (0.132)**[a]
Multiple opioids vs. Single opioid present	**−0.147 (0.065)**[b]	**−1.074 (0.072)**[a]	**−1.059 (0.058)**[a]	**−0.593 (0.067)**[a]
Any benzodiazepine present	−0.115 (0.066)	−0.065 (0.072)	0.054 (0.062)	0.026 (0.065)
Any stimulant present	−0.039 (0.069)	**−0.514 (0.095)**[a]	**−0.296 (0.084)**[a]	−0.154 (0.088)
TCA present	−0.168 (0.264)	0.006 (0.165)	−0.170 (0.131)	−0.084 (0.149)
SSRI present	−0.122 (0.173)	0.171 (0.123)	0.015 (0.109)	−0.043 (0.112)
DPH present	0.192 (0.214)	−0.223 (0.144)	−0.136 (0.135)	0.017 (0.116)
Cardiovascular disease	0.061 (0.069)	0.121 (0.069)	−0.048 (0.061)	0.009 (0.074)
Pulmonary disease	−0.025 (0.088)	−0.104 (0.077)	**−0.171 (0.070)**[b]	−0.118 (0.083)
History of substance abuse	−0.014 (0.082)	**−0.158 (0.067)**[b]	**−0.203 (0.059)**[a]	0.005 (0.064)

Bold type: $p < 0.05$.
BMI = body mass index, TCA = tricyclic antidepressant, SSRI = selective serotonin receptor inhibitor, DPH = diphenhydramine.
[a] $p \leq 0.01$.
[b] p = 0.02.

Table 5
Log Transformed Opioid Concentrations With or Without Ethanol.

Log-concentration, mean (range)	Fentanyl N = 1238	Hydrocodone N = 1018	Oxycodone N = 1562	Methadone N = 783
Ethanol concentration absent	−4.31 (−8.34, -0.48)	−2.64 (−5.30, 0.47)	−1.74 (−5.30, 2.31)	−1.06 (−4.61, 1.69)
Ethanol concentration <0.08%	−4.52 (−7.60, −1.83)	−3.06 (−6.73, 0.20)	−1.89 (−4.61, 0.72)	−1.32 (−4.61, 0.95)
Ethanol concentration ≥0.08%	−4.60 (−7.26, −1.81)	−2.76 (−5.26, 0.32)	−1.74 (−4.71, 2.08)	−1.33 (−3.00, 0.98)
Total	−4.34 (−8.34, −0.48)	−2.67 (−6.73, 0.47)	−1.75 (−5.30, 2.31)	−1.08 (−4.61, 1.69)

Table 6
Average Percent Change in Predicted Opioid Concentrations in μg/mL.

	Percent change (%)			
Factor	Fentanyl	Hydrocodone	Oxycodone	Methadone
Age, 10 years	−0.6	6.6	**7.6**	3.1
BMI, 5 units	**−9.1**	−2.7	−1.3	3.1
Male	**−16.7**	7.6	−2.7	**−16**
Ethanol concentration < 0.08% vs. Absent	−17.5	**−36.5**	−19.5	−24.7
Ethanol concentration ≥ 0.08% vs. Absent	**−27.5**	**−30.5**	−13.9	**−32.4**
Multiple opioids vs. Single opioid present	**−13.7**	**−65.8**	**−65.3**	**−44.7**
Any benzodiazepine present	−10.9	−6.3	5.5	2.6
Any stimulant present	−3.9	**−40.2**	**−25.6**	−14.3
TCA present	−15.5	0.6	−15.6	−8
SSRI present	−11.5	18.6	1.6	−4.2
DPH present	21.2	−20	−12.7	1.7
Cardiovascular disease	6.3	12.9	−4.7	0.9
Pulmonary disease	−2.5	−9.9	**−15.7**	−11.1
History of substance abuse	−1.4	**−14.6**	**−18.4**	0.5

Values in bold: $p < 0.05$.
BMI = body mass index, TCA = tricyclic antidepressant, SSRI = selective serotonin receptor inhibitor, DPH = diphenhydramine.

with statistically significant reductions in fentanyl (28%) and methadone (32%) concentrations. All four opioids were associated with statistically significantly lower predicted concentrations when multiple opioids were present, ranging from a decrease of 13.7% for fentanyl to decreases of 65–66% for hydrocodone and oxycodone.

Benzodiazepines were present in many fatalities, ranging from a low of 34% of fentanyl-related deaths to over two-thirds (68%) of hydrocodone deaths (Table 2). The presence of one or more benzodiazepines was associated with only small, non-statistically significant changes in log-transformed opioid concentrations. Stimulant presence was associated with a significant 40% reduction in hydrocodone concentrations and 26% reduction in oxycodone concentrations. A TCA was present in a fairly small percentage of the overall deaths and was associated with non-significant reductions in the opioid concentrations. Variable non-significant changes in opioid concentrations were associated with the presence of an SSRI or diphenhydramine.

Co-morbid cardiovascular disease was present in a substantial percentage of decedents overall (44%), ranging from about 36% (fentanyl) to 51% (oxycodone) (Table 2). Cardiovascular disease was associated with small, non-significant concentration changes with all opioids. Pulmonary disease was present in over 1 in 5 decedents, ranging from about 15% (fentanyl) to 26% (hydrocodone). Co-morbid pulmonary disease was associated with a statistically significant change only for oxycodone concentrations (decrease of 16%). A history of substance abuse was present in about 65% of decedents overall (82% with fentanyl vs. 49–58% with other opioids), and it was associated with statistically significant reductions in oxycodone (18%) and hydrocodone (15%) concentrations.

4. Discussion

This study demonstrates that co-ingestion of alcohol, multiple opioids or stimulants is associated with significantly decreased concentrations of commonly identified opioids in drug-related deaths. Earlier studies also found enhanced toxicity from opioid co-ingestion with alcohol or respiratory depressant drugs and that drug concentrations in polysubstance deaths were generally less than in single drug deaths [14,18,19]. However, our study more clearly defined the association of alcohol with decreased opioid concentrations, especially in the common situation when multiple drugs are involved. The analytic approach of multiple linear regression used in this study enables estimation of alcohol effects on log-opioid concentrations while accounting for the effects of other important factors, such as multiple opioid and stimulant presence and comorbidities. Alcohol was found to be involved in 17% of drug related deaths and elevated blood alcohol concentrations (BACs) were associated with reduced concentrations for most opioids involved in these deaths. Both low and high (≥0.08%) BACs were associated with significantly reduced hydrocodone concentrations; fentanyl and methadone concentrations were statistically significantly lower with the higher BACs. About 14–20% reductions were associated with oxycodone concentrations in the presence of both high and low BACs, although not statistically significant. Previous work focusing on cases with only single opioids present found significant associations of alcohol with lower concentrations of oxycodone, hydrocodone, and methadone [15], indicating that individuals could be more sensitive to opioid toxicity with alcohol co-ingestion. Another paper reported oxycodone concentrations to be lower in polysubstance deaths involving alcohol, although the presence of other drugs was a potential confounding factor [17]. These findings combined with our current results provide quantitative evidence for an enhanced toxic effect of alcohol when combined with the opioids studied.

Multiple opioid ingestion was significantly associated with lower concentrations of all four opioids in our study, likely due to enhanced respiratory depressant effects [20], providing quantitative evidence for enhanced toxicity that can result from combining opioids. Prior studies, however, have not consistently found associations between opioid concentrations in multiple substance

deaths. One study did not find a significant difference in decedent morphine concentrations when other opioid co-intoxicants were present [21], and significant differences were not found in methadone concentrations when present alone or combined with other drugs in another study [22]. No differences were also found between methadone concentrations in the presence of central nervous stimulants or respiratory depressants for a small sample of methadone-related deaths [23]. In our study, the association of opioid concentrations with multiple opioid ingestions was particularly strong for hydrocodone and oxycodone, with concentration reductions of approximately two-thirds seen when these agents were in the presence of one or more additional opioids. Fentanyl concentrations showed the smallest association (14% reduction) in single opioid vs. combined opioid deaths, perhaps due to fentanyl's greater toxicity potential in even small quantities. Acute fentanyl toxicity could also cause muscle rigidity that can further impair breathing [20]. Differences in abuse patterns, e.g., changes over time, routes of administration, might also contribute to differences seen in the associations found among the opioids in this study. Additional analyses conducted by the investigators for each of the four opioids studied found fairly consistent mean opioid concentrations over time. For hydrocodone, oxycodone and methadone (not fentanyl), there were some differences noted in the mean concentrations found with differing routes of administration (when known/documented). However, oral was the primary administration route used for these drugs (documented in 42–46% of cases) vs. the injected route (documented in 9–12% of cases), with the oral concentrations generally being higher than injected (range of 30–77% higher).

One strength of our study was the ability to also examine simultaneously the effects of benzodiazepines, the most frequent co-intoxicant found with opioids. They can be taken to enhance opioid euphoria, although the respiratory depression risk is increased and oxygen saturation is decreased [13,24]. Concurrent benzodiazepine and opioid use was found to significantly increase the risk of several adverse outcomes among U.S. veterans, including opioid and non-opioid overdose and death [25]. Higher concentrations of both opioids and benzodiazepines were found in fatal co-intoxications compared to concentrations found in persons driving under the influence [26], with the conclusion that there was a greater mortality risk with this combination. Benzodiazepines were associated with lower oxycodone concentrations in one study, although the influence of alcohol and other drugs was not accounted for [17]. A previous study found that alprazolam, but not diazepam, was significantly associated with reduced hydrocodone concentrations in the deaths. Thus, it was anticipated that benzodiazepine presence might be associated with lower opioid concentrations in this study. However this was not evident, perhaps due to potentially complex interactions among these agents [13]. In addition, our earlier study excluded cases with multiple opioids or with benzodiazepines other than alprazolam or diazepam, and did not factor in comorbidities or a history of substance abuse in the analyses [15], which make the earlier study difficult to compare directly with our current study.

Stimulants were present in about 19% of opioid-related deaths in this study, most often with fentanyl (30%) and less often with hydrocodone and oxycodone (14%). This is consistent with other reports of the increasing combination of fentanyl and stimulants such as cocaine and methamphetamine [2,27]. There is also an emerging drug use pattern of concurrent injection of methamphetamine and heroin [28]. Combined abuse of stimulants and opioids could result in enhanced cardiovascular adverse events [29], and this combination might also increase the overdose risk from opioid-induced respiratory depression once the stimulant effects subside [27]. Studies have shown that acute cocaine toxicity is enhanced in the presence of cocaethylene, an active metabolite formed in the body from cocaine and alcohol co-ingestion [11]. Our study found decreases in all four opioid concentrations associated with stimulant presence, including statistically significant hydrocodone (40%) and oxycodone (26%) reductions.

Antidepressants were present in about 13% of prescription opioid-related deaths in the U.S. during 2014–15 [30], similar to the overall percentage (11%) found in our study. Concurrent TCA, SSRI or diphenhydramine presence was not associated with statistically significant changes in any of the opioid concentrations in our study, although sample sizes were relatively small.

Several other factors were also statistically significantly associated with fentanyl, hydrocodone, oxycodone, and methadone postmortem concentrations in the present study. Age (for each 10 year increase) was associated with a relatively small increase in only oxycodone concentrations, although these incremental changes might become more important when comparing differences between relatively young (20–30 years old) and older individuals (60+ years). Obesity might increase a person's sensitivity to opioid respiratory depression [20], and it has been significantly associated with a lower lethal BAC, possibly resulting from impaired respiratory function [6]. Increases in BMI in our study were associated with small statistically significant decreases in fentanyl concentrations, although not all study decedents were obese. Relatively small, statistically significant increases in methadone and fentanyl concentrations were associated with males, but not females. In general, smaller body size might be expected to result in higher drug concentrations in females for a given exposure, as observed previously with buprenorphine [31]. However, many other pharmacokinetic and pharmacodynamic factors could affect concentrations, and differing sex sensitivity to drug actions could also impact toxicity [32].

High percentages of hypertrophy and atherosclerosis have been reported in opioid mortality cases [33], with speculation that underlying cardiovascular disease could increase the risk of hypoxia related cardiac arrest or arrhythmias from opioid toxicity. Although cardiovascular pathology was present in a substantial percentage (43%) of our study decedents, statistically significant associations were not found for the opioid concentrations studied. Co-morbid pulmonary disease was also fairly common in our study. Underlying impairment in respiratory function might be expected to enhance opioid-induced respiratory depression. The predicted oxycodone concentrations were found to be slightly but statistically significantly lower with pulmonary disease, with only slight non-significant associations present for the other opioids. After the initial modeling was conducted, hepatic disease (including hepatitis, cirrhosis, liver disease [alcoholic or not otherwise specified], hepatic necrosis, or hepatic failure) was considered as a possible variable that could affect opioid metabolism and the resulting opioid concentrations observed. Thus, the modeling was subsequently repeated adding in presence or absence of hepatic disease as a covariate. No statistically significant associations (p values from 0.10–0.41) were found for any of the four opioids studied, nor were any of the other associations significantly affected.

A history of substance abuse is fairly common with opioid overdose. It might be anticipated that such a history would be associated with higher opioid concentrations due to potential tolerance development with chronic use, and a need to ingest greater amounts of opioids to achieve the same effect [34]. However, loss of opioid tolerance after a period of abstinence (e.g., incarceration, receiving medication-assisted treatment) might increase the risk of overdose from a given intake [35]. Statistically significant reductions in hydrocodone and oxycodone concentrations were found in our study to be associated with a history of substance abuse. The reasons for this are unclear since details

about a decedents' prior substance abuse history were not available.

The percent changes in predicted opioid concentrations found in this study can be multiplicative and provide a quantitative illustration of the dangers from polysubstance ingestions. For example, the presence of one or more additional opioids was associated with a significant, almost two-thirds reduction in hydrocodone concentrations compared to hydrocodone alone (Table 6). Alcohol ingestion might be associated with an additional one-third reduction, resulting in a predicted hydrocodone concentration that is only 22% of the concentration expected from hydrocodone alone in deaths.

There are potential limitations associated with this study. It is difficult to clearly determine whether a death was unintentional or a suicide; thus, the number of suicides related to drug overdose deaths are likely underestimated [36]. Case data might be missing or incorrectly entered in decedent files or the FDD, although data were checked for inconsistencies or missing values with follow-up to the extent possible. Several deaths were excluded from analyses due to missing femoral or subclavian drug concentrations, although these accounted for only 14.5% of the overall data sample. Substantial interpatient variability exists in postmortem drug concentrations, which could make it difficult to identify some opioid concentration associations, especially for substances such as TCAs, SSRIs, and diphenhydramine that were less commonly identified. Interpreting postmortem drug concentrations can also be difficult due to potential postmortem redistribution (PMR). Only peripheral blood was used in all analyses in the current study to lessen the extent to which PMR might affect the findings. In addition, given the large number of cases studied with varying postmortem intervals, it is likely that PMR would affect the comparisons (e.g., presence vs. absence) similarly.

Variations in analytical methods and postmortem sampling, or complex in vivo drug effects can also affect the associations, or lack thereof, between opioid concentrations and the presence of co-intoxicants such as benzodiazepines in drug-related deaths. When identifying whether multiple opioids were present in the deaths, it is possible that a few as yet unknown fentanyl analogs or other illicit opioid analogs were not detected and thus not considered when classifying deaths as involving single vs. multiple opioids. There might also have been some data complexities when other opioid concentrations were present due to our use of a factor of multiple opioid presence or absence. However, we elected to examine presence/absence of multiple opioids because of varying morphine equivalents and resulting concentrations of the individual opioids present. Finally, although efforts were made to identify cardiovascular or respiratory co-morbidities or a history of substance abuse from the decedents' files, which included multiple sources of information, some of these conditions could have been missed.

5. Conclusions

These findings provide additional, quantitative evidence for an enhanced overdose risk from alcohol and opioid co-ingestion, with several fairly large, significant associations found between opioid concentrations observed and factors such as multiple opioid or alcohol presence. Multiple regression modeling enabled us to examine the importance of several factors simultaneously to determine the association of alcohol and opioid concentrations. Our findings have important clinical implications for those treating or working with persons who have substance use disorders and for those prescribing opioids, especially in the elderly, those with comorbidities, and chronic pain patients. These patients should be strongly advised that even small amounts of alcohol use can potentially increase the risk of fatal overdose. The risks of mixing multiple drugs and/or alcohol are considerable and should be considered in any programs designed to reduce opioid-related deaths. Documenting all potential co-intoxicants in drug-related deaths is also essential to increase knowledge of possible interactions and to maximize drug overdose prevention and treatment efforts.

Contributors

ZD, MA and TH formulated the research question and designed the study. MA, TH and ZD drafted the initial manuscript. ZD conducted statistical analyses and DLL provided statistical expertise. MA and ZD designed the tables and MA incorporated suggestions from all co-authors. MA, GS, JK and AK contributed to the conception of the analyses, interpretation of the results and manuscript writing. All authors had full access to all of the data and approved the final manuscript.

Conflict of interest

No conflict declared.

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CRediT authorship contribution statement

Zheng Dai: Conceptualization, Formal analysis, Methodology, Writing - original draft, Writing - review & editing. **Marie A. Abate:** Conceptualization, Data curation, Funding acquisition, Methodology, Supervision, Validation, Writing - original draft, Writing - review & editing. **D. Leann Long:** Formal analysis, Methodology, Writing - review & editing. **Gordon S. Smith:** Data curation, Funding acquisition, Methodology, Supervision, Validation, Writing - review & editing. **Theresa M. Halki:** Conceptualization, Writing - original draft. **James C. Kraner:** Data curation, Supervision, Validation, Writing - review & editing. **Allen R. Mock:** Data curation, Supervision, Validation, Writing - review & editing.

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