AH-7921 (3,4-dichloro-\(N\)-(1-dimethylamino)cyclohexylmethyl) benzamid) is a designer opioid with \(~80\%\) of morphine's \(\mu\)-agonist activity. Over a 6-month period, we encountered nine deaths where AH-7921 was involved and detected in blood from the deceased. Shortly after the last death, on August 1 2013, AH-7921 was scheduled as a narcotic and largely disappeared from the illicit market in Sweden. AH-7921 was measured by a selective liquid chromatography–MS-MS method and the concentrations of AH-7921 ranged from 0.03 to 0.99 \(\mu\)g/g blood. Six of our cases had other drugs of abuse on board and most had other medications such as benzodiazepines, antidepressants and analgesics. However, the other medicinal drugs encountered were present in postmortem therapeutic concentrations and unlikely to have contributed to death. In addition to the parent compound, we identified six possible metabolites where two \(N\)-demethylated dominated and four mono-hydroxylated were found in trace amounts in the blood. In conclusion, deaths with AH-7921 seem to occur both at low and high concentrations, probably a result of different tolerance to the drug. Hence, it is reasonable to assume that no sharp dividing line exists between lethal and non-lethal concentrations. Further, poly-drug use did not seem to be a major contributing factor for the fatal outcome.

Case descriptions
Over a 6-month period, we encountered nine deaths where the \(\mu\)-agonist AH-7921 was involved and detected in blood from the deceased.

Case 1
A 27-year-old male known drug addict was found dead at home laying on the kitchen floor. In the apartment, there were powders and equipment for intravenous injections, indicating an ongoing abuse of drugs. He was seen alive 2 days before he was found dead. Autopsy revealed pulmonary edema, a tablet remnant in the stomach together with fresh and older needle marks on the right arm. The weight of the lungs was 1,211 g. Tryptase level was high, 528 \(\mu\)g/L.

Case 2
A 26-year-old male was found dead on a sofa at his apartment. Empty beer cans but no pharmaceutical drugs were found at the scene. He had psychiatric problems for 3 years recently, but according to the relatives had not been depressed. When last contacting the psychiatry care, 5 days before he was found dead, he said he felt normal, was well off and was taking his medications. Autopsy revealed pulmonary edema, and signs of aspiration pneumonia. The weight of the lungs was 1,712 g.

Case 3
A 24-year-old male known drug addict was found dead at home laying on the kitchen floor. He had psychiatric problems for 3 years recently, but according to the relatives had not been depressed. When last contacting the psychiatry care, 5 days before he was found dead, he said he felt normal, was well off and was taking his medications. Autopsy revealed pulmonary edema, and signs of aspiration pneumonia. The weight of the lungs was 1,712 g.

Case 4
A 45-year-old male known drug addict was found dead at home laying on the kitchen floor. He had psychiatric problems for 3 years recently, but according to the relatives had not been depressed. When last contacting the psychiatry care, 5 days before he was found dead, he said he felt normal, was well off and was taking his medications. Autopsy revealed pulmonary edema, and signs of aspiration pneumonia. The weight of the lungs was 1,712 g.

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**Case 5**
A 26-year-old male with a history of psychiatry disease, narcolepsy and ADHD was found dead in his bed. Autopsy revealed pulmonary congestion. The weight of the lungs was 1,254 g.

**Case 6**
A 34-year-old male was found dead on a sofa at his apartment. He had not been seen for 4 days. Several pharmaceuticals as well as designer drugs were found at the scene. He was a known drug addict and was hospital treated due to intoxication the previous year. Autopsy findings were unremarkable. Hair analysis suggested previous use of tramadol but was negative for AH-7921. The weight of the lungs was 792 g.

**Case 7**
A 27-year-old male was found unresponsive in his bed by his girlfriend. According to his relatives, he had suffered from psychiatric problems for a long time and had been using drugs. Autopsy revealed extended lungs and bronchopneumonia. The weight of the lungs was 2,146 g.

**Case 8**
A 22-year-old male was found unresponsive sitting in his room. He was a known drug user and had been depressed. Autopsy revealed pulmonary congestion, acute bronchitis, pneumonia and brain edema. The weight of the lungs was 1,256 g.

**Case 9**
A 22-year-old male was found by the personnel at a treatment facility. Beside him there was Lyrica tablets and a white powder labeled 'AH-7921'. Autopsy revealed some tablets in the stomach, possible needle marks in both arms, brain edema and pulmonary congestion. The weight of the lungs was 1,590 g.

**Experimental**
Routine postmortem toxicology was performed in femoral blood using a targeted screening for pharmaceuticals and drugs of abuse with liquid chromatography time-of-flight (LC-TOF) technology (26). An in-house database comprising nearly 550 drugs was used for determination of primary target compounds relevant for postmortem toxicology. Data were extracted by the 'Find by Formula' algorithm with a match tolerance for mass error of ±10 ppm, retention time deviation ±0.15 min and area of ≥30,000 counts. Identification was based on scoring of retention time, accurate mass measurement and isotopic pattern (mass, abundance and spacing). Medications and drugs of abuse screened positive with LC-TOF were quantified in femoral blood using in house methods. Analysis of alcohols and acetone were performed in blood and urine or vitreous humor using head space gas chromatography with flame ionization detection.

**Quantification of AH-7921**
AH-7921 was obtained from Cayman Chemical Company (Ann Arbor, MI, USA) and the internal standard EDDP-d3 was obtained from Cerilliant (Round Rock, TX, USA).

To 0.5 gram of blood 50 μL of internal standard (1.0 μg/mL of EDDP-d3 in methanol) was added and the blood was precipitated with 1.0 mL of 0.075% formic acid in acetonitrile: ethanol (90:10). After mixing, the sample was centrifuged for 10 min at 5,000 rpm and a 100-μL aliquot was transferred to an autosampler vial and 3 μL were injected.

The analysis was performed on an AT6460 triple quadrupole instrument using an electrospray interface. The analytical column was an Agilent Zorbax Eclipse Plus C18 (2.1 × 50 mm) with 1.8 μm particle size. Mobile phase A was 0.05% formic acid in 10 mM ammonium formate and phase B was 0.05% formic acid in methanol run in a linear gradient from 5% B to 70% B within 3 min at a total flow of 0.5 mL/min. For AH-7921, two transitions were measured (329/284 and 329/173) and one transition for the internal standard (282/235).

Validation was performed as proposed by Peters et al. for methods used in case reports (27). Ten postmortem blood samples with varying degrees of decomposition and 10 samples from living subjects without addition of internal standard, a blood sample with internal standard as well as blood samples fortified with 45 different drugs of abuse were analyzed to investigate the selectivity. Matrix effects were investigated qualitatively by infusion of analyte when injecting negative samples (N = 4). Calibration model was determined analyzing triplicates at eight levels (0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 0.7 and 1.0 μg/g). A mean within 10% of the target value was considered acceptable for

<table>
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<tbody>
<tr>
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<td>34</td>
<td>February, 2013</td>
<td>0.03*</td>
<td>Ethanol 0.11*</td>
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BMI, Body mass index.
*Heart blood.
1Ethanol reported at g/L.
establishing the calibration range. Five replicates at three levels (0.02, 0.20 and 0.70 μg/g) were used to estimate repeatability and accuracy.

**Metabolite investigations**

LC-QTOF analysis was performed on an Agilent 6540 quadrupole TOF mass spectrometer (Agilent Technologies, Kista, Sweden) equipped with a JetStream interface in combination with an Agilent 1290 Infinity UHPLC instrument (Agilent Technologies). Mobile phase A consisted of 0.05% formic acid in 10 mM ammonium formate and phase B of 0.05% formic acid in acetonitrile. Separation was achieved within 12 min by a linear gradient chromatography at a 0.5 mL/min flow rate on a ACQUITY UPLC HSS T3 column, 150 × 2.1 mm, 1.8 μm (Waters, Stockholm, Sweden) maintained at 60°C. Ions were generated in positive ion single MS mode (2 GHz), $m/z$ range 50–1,000. Ion source parameters: drying gas 6 L/min at 300°C and sheath gas 10 L/min at 375°C. Data acquisition were performed using MassHunter Acquisition B.04.00 and evaluation was performed using MassHunter Qualitative Analysis B.06.01. Fragmentation investigation on AH-7921 was done by flow injection analysis on a pure standard solution (0.1 μg/mL in methanol). Fragmentation data were evaluated in MassHunter Molecular Structure Correlator B.05.00 software.

**Results and discussion**

The quantitation method for AH-7921 proved simple and accurate. The selectivity tests revealed no interfering peaks at the retention time of the analyte or internal standard. The qualitative matrix effect studies showed matrix effects at 1.75 min and
1.95 min in putrefied autopsy samples but no matrix effects after 1.95 min. The retention time of the internal standard was 2.11 min and that of AH-7921 2.23 min. Calibration model was determined to be best fitted to a quadratic function using 1/X weighting and the range was determined to 0.01–1.0 μg/g blood. The repeatability tests showed coefficients of variation of 5% at 0.02 and 0.20 μg/g, and 1% at 0.70 μg/g. Accuracy was 105–115%, 94–103% and 101–104%, respectively.

In this case series, eight of the deceased were signed out as intoxications and one aspiration caused by intoxication. In four cases, the manner of death was uncertain and in five it was accidental. Table I shows the toxicological findings and the cause and manner of death for each case. The AH-7921 concentrations ranged from 0.03 to 0.99 μg/g blood but there was no case that presented with only AH-7921. In comparison to Vorce et al. (20) the concentrations in our nine cases were much lower but still the pathologist considered these deaths attributed to AH-7921 alone or in combination with other drugs. The signs of opioid toxicity are few; however, pulmonary edema and heavy lungs are commonly seen in opioid overdose deaths even though the mechanisms are incompletely understood (28). The lung weights in seven of the present cases were higher than the reference range of 1.142 g (29) which is compatible with, but not conclusive for, acute intoxication with the μ-agonist AH-7921. In one case, the lungs were donated prior to autopsy and the weights could not be measured.

Deaths from acute overdose of opioids often present with a wide range of blood concentrations and it has been suggested that rather than a lethal concentration, there are other factors that contribute to the death. Factors include poly-drug use, tolerance, allergic reactions and contributing pathology.

Case 1, for example, had only a therapeutic concentration of gabapentin present in femoral blood together with an AH-7921 concentration of 0.81 μg/g. The parapneumia at the scene suggested that he could have injected AH-7921 and died suddenly. This was also in agreement with high levels of tryptase that might indicate an acute allergic reaction known to occur in deaths related to intravenous injection of narcotics (30).

Poly-drug use has been postulated as a major risk factor in opioid deaths, especially for buprenorphine as very few buprenorphine-only fatal intoxications have been reported (31–33). Six of the cases had other drugs of abuse on board and medications such as benzodiazepines, antidepressants and analgescics were also commonly found. Even though they were found in therapeutic concentrations their presence may have contributed to death. Pyrazolam and 3-methylmethcathinone were not quantified in cases 4, 5 and 8 and their contribution to death cannot be disregarded.

Another major risk factor in opioid deaths is reduced drug tolerance (29, 34). Due to the scarce literature on AH-7921, we could only speculate on this matter when interpreting the concentrations of the present designer opioid. Even if a majority of the cases were known drug addicts, no information of previous use of AH-7921 was available. Interestingly, only one case presented with other opioids in blood, namely tramadol and codeine (Case 4). In Case 6, hair was analyzed and pointed towards previous use of tramadol but not AH-7921. The other cases might have been naive opioid users which could have contributed to the fatal outcome. In summary, the interpretation of emerging opioids such as AH-7921 seems as difficult as the more established ones.

Figure 1 shows the MS–MS spectra from AH-7921 and chromatograms from Case 5. Table II shows peak information from Case 5 where several tentative metabolites (M1–M6) were found in the femoral blood. Table III shows the relative abundance of the six tentative metabolites for each case. There was a trend towards more metabolites at lower AH-7921 concentrations, even though the actual concentrations could not be determined. It also illustrates the confirmed delayed death (Case 3) with the lowest AH-7921 concentrations but the most abundant M1 and M2. Both Vorce et al. and Soh and Elliott suggested metabolites that were N-demethylated and those were the most abundant metabolites in our cases too (20, 25).

The fragmentation of AH-7921 as well as M1 and M2 in our LC-QTOF method was in agreement with that presented by Soh and Elliott using the same analytical technique. There was no change in fragmentation pattern between the parent compound and N-demethylated metabolites as the charge was on the amide side of the molecule rather than the amine side. In addition to M1 and M2, we also identified four additional mono-hydroxylated metabolites with retention times on both sides of the parent...
AH-7921. Unfortunately, they all had very low abundance, ~1% of the AH-7921 peak area and no MS–MS spectra could be obtained.

Hydroxylated metabolites may be conjugated with glucuronic acid but when investigated we found no peaks corresponding to the hydroxyl metabolite glycones.

Over a 6-month period, we encountered nine deaths where the µ-agonist AH-7921 was involved and detected in blood from the deceased. Shortly after the last death, on August 1, 2013, AH-7921 was scheduled as a narcotic and disappeared from the illicit market in Sweden.

Conclusions
In conclusion, deaths with AH-7921 seem to occur both at low and high concentrations, probably a result of different tolerance to the drug. Hence, it is reasonable to assume that no sharp dividing line exists between lethal and non-lethal concentrations. Further, poly-drug use did not seem to be a major contributing factor for the fatal outcome. The interpretation of emerging opioids such as AH-7921 seems equally difficult as the more established ones and should include a comprehensive drug screening, including hair analysis to investigate poly-drug use and possible tolerance to opioids.

References


