



Isotonitazene

Report on the risk assessment of *N,N*-diethyl-2-[[4-(1-methylethoxy)phenyl]methyl]-5-nitro-1*H*-benzimidazole-1-ethanamine (isotonitazene) in accordance with Article 5c of Regulation (EC) No 1920/2006 (as amended)

About this series

EMCDDA Risk Assessments are publications examining the health and social risks of individual new psychoactive substances.

The Risk Assessment Report consists of an analysis of the scientific and law enforcement information available on the new psychoactive substance under scrutiny and the implications of placing it under control. It is the outcome of a meeting convened under the auspices of the EMCDDA Scientific Committee.

This process is part of a three-step procedure involving information exchange/early warning, risk assessment and decision-making in the framework of Regulation (EC) 1920/2006 (as amended by Regulation (EU) 2017/2101).

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Statement regarding the United Kingdom

The reference period for this risk assessment report includes 2019 and 2020 (up to the time of writing). The United Kingdom left the EU as of 1 February 2020. However, during the transitional period, the United Kingdom continues to participate in the EU Early Warning System on new psychoactive substances. Unless stated otherwise, for the purpose of this report, the term 'Member States' shall include the United Kingdom.

Foreword

This publication presents the data and findings of the risk assessment on isotonitazene (*N,N*-diethyl-2-[[4-(1-methylethoxy)phenyl]methyl]-5-nitro-1*H*-benzimidazole-1-ethanamine), carried out by the extended Scientific Committee of the EMCDDA on 26 May 2020.

The Risk Assessment Report, which was submitted to the European Commission on 29 May 2020, examines the health and social risks of the drug, information on international trafficking and the involvement of organised crime, as well as a consideration of the potential implications of subjecting the drug to control measures. Isotonitazene is the first new psychoactive substance to be risk assessed under the Article 5c of Regulation (EC) No 1920/2006 (as amended by Regulation (EU) 2017/2101).

On the basis of the Risk Assessment Report the Commission adopted delegated act in order to add isotonitazene in to the definition of a drug in the Annex of the Council Framework Decision 2004/757/JHA (as amended by Directive (EU) 2017/2103). This decision was adopted in the final stage of the three-step process — early warning, risk assessment and control of new psychoactive substances — established by Regulation (EC) 1920/2006 (as amended) and Council Framework Decision 2004/757/JHA (as amended).

Regulation (EC) 1920/2006 (as amended) and Council Framework Decision 2004/757/JHA (as amended) allow the EU institutions and Member States to act on all new and potentially threatening narcotic and psychotropic drugs which appear on the European drug scene, with the EMCDDA and Europol, in collaboration with their respective networks playing a central role in the early detection of such substances as well as the harms caused by their use — information that underpins risk assessment, and, ultimately, decision-making.

In this respect we would like to acknowledge the essential role played by the respective networks in the Member States — the Reitox national focal points — in collecting and providing national data. We would also like to acknowledge the role of Europol and Europol National Units, the European Medicines Agency (EMA) and the national competent authorities responsible for medicinal products, the European Chemicals Agency (ECHA), the European Centre for Disease Prevention and Control (ECDC) and the European Food Safety Authority (EFSA) that provided valuable input to the report, thus guaranteeing its truly multidisciplinary nature.

Finally, we would like to thank all the participants in the risk assessment process for the high quality of work carried out. This procedure occurred under challenging circumstances, amidst the coronavirus (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The resulting report is a valuable contribution at European level, and provides clear support to political decision-making.

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EMCDDA Initial Report on *N,N*-diethyl-2-[[4-(1-methylethoxy)phenyl]methyl]-5-nitro-1*H*-benzimidazole-1-ethanamine (isotonitazene)

On 20 February 2020, the EMCDDA assessed the existing information on *N,N*-diethyl-2-[[4-(1-methylethoxy)phenyl]methyl]-5-nitro-1*H*-benzimidazole-1-ethanamine (commonly known as isotonitazene), based on the following criteria: (1) reports of health problems; (2) reports of social problems; (3) reports of seized material; (4) pharmacological and toxicological properties and analogy with better-studied substances; and, (5) potential for further spread.

The EMCDDA concluded that the assessment gave rise to concerns that isotonitazene may pose health or social risks at Union level, and, consequently, determined that an initial report should be produced.

On 3 April 2020, the EMCDDA submitted to the Commission and Member States an initial report on the new psychoactive substance isotonitazene, in accordance with Article 5b of the Regulation (EC) 1920/2006 (as amended). The report concluded that the health and social risks, caused by the use of, the manufacture of, and traffic in isotonitazene, as well as the involvement of organised crime and possible consequences of control measures, could be thoroughly assessed through a risk assessment procedure as foreseen by Article 5c of the Regulation (EC) 1920/2006 (as amended).

The full text of the Initial Report can be found at:

https://www.emcdda.europa.eu/publications/initial-reports/isotonitazene_en

Risk Assessment Report on a new psychoactive substance: *N,N*-diethyl-2-[[4-(1-methylethoxy)phenyl]methyl]-5-nitro-1*H*-benzimidazole-1-ethanamine (isotonitazene)

Introduction

New psychoactive substances can cause serious cross-border threats to health. In Europe, Regulation (EC) No 1920/2006 of the European Parliament and of the Council of 12 December 2006 on the European Monitoring Centre for Drugs and Drug Addiction (recast) (as amended) (hereafter the 'Regulation') and Council Framework Decision 2004/757/JHA of 25 October 2004 laying down minimum provisions on the constituent elements of criminal acts and penalties in the field of illicit drug trafficking sets up a three-step legal framework of early warning, risk assessment and control measures that allows the EU to rapidly detect, assess and respond to the public health and social risks caused by new psychoactive substances. The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) is responsible for the first two steps in this system, namely operating the EU Early Warning System on new psychoactive substances (EU EWS) in close cooperation with Europol, and conducting risk assessments. The European Commission is responsible for proposing control measures. Thus, the legal framework allows the institutions of the EU and the Member States to act on all new psychoactive substances (NPS) that appear on the European drug market.

In accordance with Article 5a of the Regulation, *N,N*-diethyl-2-[[4-(1-methylethoxy)phenyl]methyl]-5-nitro-1*H*-benzimidazole-1-ethanamine (commonly known as isotonitazene) was formally notified as an NPS by the EMCDDA on behalf of Belgium on 26 August 2019. The notification was based on the identification of the substance in a collected sample of powder that was a test purchase from a private online vendor and which was received on 1 July 2019 ⁽¹⁾.

Based on the information reported to the EU EWS, and, in accordance with Article 5a of the Regulation, on 20 February 2020, the EMCDDA assessed the existing information on isotonitazene. The EMCDDA concluded that the assessment gave rise to concerns that isotonitazene may pose health or social risks at EU level, and, consequently, determined that an initial report should be produced in accordance with Article 5b of the Regulation. The initial report was submitted to the Commission and Member States on 3 April 2020. Based on the findings of the initial report, on 17 April 2020, the Commission requested that the EMCDDA carry out a risk assessment on isotonitazene in accordance with Article 5c of the Regulation.

This risk assessment report presents the summary findings and the conclusion of the risk assessment carried out by the Scientific Committee of the EMCDDA on isotonitazene. The report is intended for policymakers and decision-makers in the institutions of the EU.

The report has been prepared and drafted in accordance with the requirements of Article 5c of the Regulation as well as the conceptual framework and the procedure set out in the EMCDDA risk

⁽¹⁾ However, based on information subsequently reported to the EMCDDA concerning a seizure made by police in Estonia, isotonitazene has been available on the European drug market since at least April 2019.

assessment operating guidelines. It is written as a stand-alone document, which presents a summary of the information considered during the detailed assessment of the scientific and law enforcement information available at this time. The conclusion section of the report summarises the main issues addressed and reflects the opinions held by the members of the Scientific Committee. A list of the information resources considered by the Scientific Committee, including a detailed technical report on isotonitazene prepared by the EMCDDA (Annex 1), is provided below.

In accordance with Article 5c of the Regulation, the meeting to assess the risks of isotonitazene was convened under the auspices of the Scientific Committee of the EMCDDA with the participation of five additional experts designated by the Director of the EMCDDA, acting on the advice of the Chairperson of the Scientific Committee, chosen from a list of experts approved by the Management Board of the EMCDDA. The additional experts were from scientific fields that were either not represented or not sufficiently represented in the Scientific Committee, and whose contribution is necessary for the balanced assessment of the risks posed by isotonitazene. A further six experts were observers to the risk assessment: two experts from the Commission, two experts from the EMCDDA, one expert from Europol, and one expert from the European Medicines Agency (EMA). The meeting took place on 26 May 2020. Owing to the ongoing response to the coronavirus (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the meeting was conducted by videoconference and hosted by the EMCDDA.

The risk assessment was carried out on the basis of information provided to the Scientific Committee by the Member States, the EMCDDA, Europol, the EMA, the European Centre for Disease Prevention and Control (ECDC), the European Chemicals Agency (ECHA) and the European Food Safety Authority (EFSA). A list of the Scientific Committee members, observers and other participants attending the risk assessment meeting is annexed to this report (Annex 2).

For the risk assessment, the Scientific Committee considered the following information resources:

- the EMCDDA technical report on *N,N*-diethyl-2-[[4-(1-methylethoxy)phenyl]methyl]-5-nitro-1*H*-benzimidazole-1-ethanamine (isotonitazene) (Annex 1);
- the EMCDDA initial report on the new psychoactive substance *N,N*-diethyl-2-[[4-(1-methylethoxy)phenyl]methyl]-5-nitro-1*H*-benzimidazole-1-ethanamine (isotonitazene);
- open source information, including scientific articles, official reports, grey literature, internet drug discussion forums and related websites (hereafter 'websites for people who use drugs');
- additional information provided during the course of the risk assessment meeting by the participants;
- EMCDDA operating guidelines for the risk assessment of new psychoactive substances;
- Regulation (EC) No 1920/2006 of the European Parliament and of the Council of 12 December 2006 on the European Monitoring Centre for Drugs and Drug Addiction (recast) (as amended); and
- Council Framework Decision 2004/757/JHA of 25 October 2004 laying down minimum provisions on the constituent elements of criminal acts and penalties in the field of illicit drug trafficking (as amended).

Background

During the mid-1950s, attempts to develop better and safer opioid analgesics led to the discovery of a series of 2-benzylbenzimidazole compounds with levels of analgesic potency several orders of magnitude higher than that of morphine. This group of structurally distinct opioid analgesics includes isotonitazene, the subject of this risk assessment report, as well as etonitazene and metonitazene,

which are closely related homologues, and clonitazene. Both etonitazene and clonitazene are controlled under the United Nations Single Convention on Narcotic Drugs of 1961 because of the potential risks they pose to public health, and, more specifically, because of their ability to produce morphine-like adverse effects, to suppress abstinence phenomena of a known morphine addiction and to sustain morphine addiction in experimental models. None of these 2-benzylbenzimidazoles has become an authorised medicine.

Although the high analgesic potency renders the manufacture of this series of compounds attractive to clandestine chemists, until the first identification of isotonitazene in 2019, when it was first identified on the illicit drug market, only etonitazene had been sporadically encountered on the drug market. As of 26 May 2020, isotonitazene is the only substance from the 2-benzylbenzimidazole series of opioid analgesics notified to the EMCDDA as an NPS.

Similar to other types of opioid analgesics such as morphine and fentanyl, the 2-benzylbenzimidazole compounds, including isotonitazene, produce most of their effects by activating the μ -opioid receptors (MOPs) in the central nervous system (CNS). The acute effects of this include euphoria, relaxation, analgesia (a reduced ability to feel pain), sedation (inducing a state of calm or sleep), bradycardia (slowing of the heart), hypothermia (dangerously low body temperature) and respiratory depression (slowing down of breathing). It is this last effect that poses the greatest danger to users, as, owing to the apparently high potency of some of these compounds, sub-milligram doses may cause life-threatening poisoning from respiratory depression. Left untreated, this can lead to respiratory arrest (cessation of breathing) and death. Although the available information is limited, the 2-benzylbenzimidazole opioids, including etonitazene and isotonitazene, are likely to have an abuse liability and dependence potential, similar to other opioid analgesics.

Isotonitazene has been available on the drug market in Europe since at least April 2019. It is important to highlight that, as isotonitazene has emerged on the drug market only recently, no formal epidemiological studies have been conducted, which limits understanding of the frequency and patterns of use of the substance. Related to this, the presence of isotonitazene on the drug market and its involvement in serious adverse events (such as acute poisonings presenting to hospital emergency rooms and medico-legal death investigations) may be undetected because the substance is not routinely screened for in some forensic and toxicology laboratories in Europe. Therefore, the presence of isotonitazene on the drug market may be undetected in some areas. Analytical sensitivity related to the analysis of biological samples from serious adverse events may also be a potential issue, as the concentration of isotonitazene or its metabolites can be at sub-nanogram level (similar to the highly potent opioid carfentanil), which highlights a need for increased analytical scrutiny when testing for the substance. In addition, in some areas not all laboratories are part of national early warning systems; for this reason, the identification of isotonitazene may go unreported to the Reitox national focal points, and, as a result, to the EMCDDA. Finally, it is also important to note that, in some settings, the ongoing COVID-19 pandemic may have reduced the capacity of early warning systems, including forensic science and toxicology laboratories, to detect and report events involving isotonitazene, or, for that matter, any other NPS.

Chemical and physical properties and the methods and precursors used for manufacture

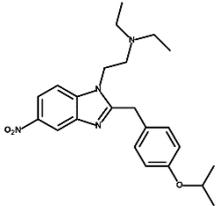
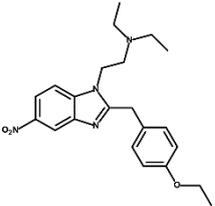
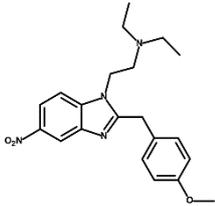
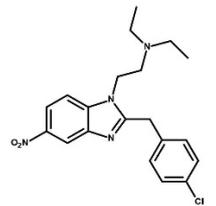
Chemical and physical properties

Isotonitazene belongs to the 2-benzylbenzimidazole group of opioid analgesics. In particular, it is a 5-nitro-2-benzylbenzimidazole derivative. This group also includes the closely related homologues, etonitazene and metonitazene, as well as clonitazene.

Isotonitazene differs from etonitazene and metonitazene in the substitution at the *para*-position of the benzyl moiety, which is an isopropoxy group in isotonitazene, an ethoxy group in etonitazene and a methoxy group in metonitazene. Isotonitazene differs from clonitazene in the replacement of the chloro halogen atom with the ethereal isopropoxy group. The *n*-propoxy isomer, protonitazene, is also known. The chemical structures, molecular formulae and molecular weights of these compounds are provided in Figure 1.

FIGURE 1

Chemical structure, molecular formula and molecular weight of isotonitazene, etonitazene, metonitazene and clonitazene.

Isotonitazene	Etonitazene	Metonitazene	Clonitazene
			
Molecular formula: C ₂₃ H ₃₀ N ₄ O ₃	Molecular formula: C ₂₂ H ₂₈ N ₄ O ₃	Molecular formula: C ₂₁ H ₂₆ N ₄ O ₃	Molecular formula: C ₂₀ H ₂₃ ClN ₄ O ₂
Molecular weight: 410.51	Molecular weight: 396.48	Molecular weight: 382.46	Molecular weight: 386.88

The free base and salts of isotonitazene are solids. Identifications of isotonitazene reported to the EMCDDA include both the free base and the hydrochloride salt.

The measured melting point for isotonitazene hydrochloride salt is 172–173°C.

Isotonitazene is lipophilic (calculated log P = 4.85).

Isotonitazene, as both the free base and the hydrochloride salt, is soluble in methanol and in dimethyl sulfoxide (DMSO). Although no experimental data are available, the salts of isotonitazene, similar to those of etonitazene, are expected to be sufficiently water-soluble for the injectable administration of effective doses.

No studies have examined the stability and reactivity of isotonitazene. As a base, the substance readily forms salts with inorganic or organic acids. Chemical reducing agents are expected to convert the nitro group into an amino group.

The analytical identification of isotonitazene in physical and biological samples is possible using several analytical techniques. These include chromatographic and mass-spectrometric techniques. Certified analytical reference material is available for isotonitazene and for other similar substances, such as protonitazene. The availability of analytical reference material is important for correct identification and for facilitating the quantification of isotonitazene in physical and biological samples. Isotonitazene is not expected to give a positive response to immunoassays developed for opioids.

Methods and precursors used for manufacture

There is no information on the actual manufacturing methods or chemical precursors used to synthesise the isotonitazene that has been identified on the drug market in Europe. Information from one seizure made by customs suggests that at least some isotonitazene on the market in Europe has been supplied from China. Although the synthetic routes reported in the scientific literature involve three or four steps, depending on precursor availability, they are straightforward and make use of common manufacturing equipment. The starting materials for either synthetic route are readily available. The multi-step production of isotonitazene requires basic knowledge of and experience in synthetic organic chemistry.

Pharmaceutical forms

Information on the pharmaceutical forms available for isotonitazene is limited. Isotonitazene can be administered orally as a powder, as tablets or as a solution; it can also be administered intranasally or sublingually via spray or snorted (insufflated), inhaled by vaporising e-liquid solutions ('vaping'), inhaled by smoking or vaporising the 'free base', and injected.

Given the illicit nature of the trade in isotonitazene, the composition of products containing isotonitazene is likely to vary over time and place, as well as based on the specific location in the drug supply chain from which the product was obtained (e.g. from the manufacturer, wholesaler, retailer or at street-level illicit opioid markets). In Europe, isotonitazene has been seized by law enforcement predominantly in powder form; in two cases it has also been seized as a liquid. Information on the amount of isotonitazene present in seized powders (chemical purity) has not been reported; however, information from police seizures in Estonia noted the presence of 'common sugars' in at least some of the samples (not specified further), while one seizure made by police in Latvia also contained fentanyl. Results from the analysis of a collected sample of isotonitazene in powder form that was purchased from an online vendor noted that isotonitazene was estimated to be of a high purity.

Pharmacological and toxicological properties

Pharmacological properties

There is limited information on the pharmacological properties of isotonitazene. Although isotonitazene has not been formally studied in humans, a recent study has demonstrated that it is a highly potent, full MOP agonist *in vitro*, while an older study has demonstrated that it has potent morphine-like centrally mediated analgesic effects in an animal model of experimentally induced pain. As a result of its lipophilicity, isotonitazene is expected to be rapidly absorbed and to readily cross the blood–brain barrier. Taken together, this information suggests that isotonitazene will also act as an opioid analgesic in humans. The major pharmacological effects of such opioid analgesics, including their analgesic activity, are due to their activation of the MOP receptor. In addition to their analgesic properties, a notable feature associated with opioid analgesics is that they cause dose-dependent respiratory depression, which in overdose can be life-threatening. Other opioid effects include miosis,

sedation, bradycardia, hypothermia, constipation, physical dependence and changes in mood (e.g. euphoria).

According to preclinical studies with the related substance etonitazene, naloxone antagonises the effects of the opioid. In an early human clinical trial, respiratory depression and coma induced by metonitazene were antagonised by a prompt injection of nalorphine.

There is limited information on the pharmacokinetics, including half-life and potentially bioactive metabolites, of isotonitazene.

No information is available on any potential activity of isotonitazene at other pharmacological targets.

Toxicological properties

The toxicological properties of isotonitazene have not been studied. Despite the lack of experimental data, observations that antinociceptive potency in general correlates with acute toxicity within the 2-benzylbenzimidazole group of opioid analgesics — including for etonitazene or metonitazene, which are both closely related homologues of isotonitazene — suggest that the acute toxicity of isotonitazene, at least in animal models, may be much greater than morphine and similar to that estimated for etonitazene or metonitazene.

Health risks

There is limited information on the health risks posed by isotonitazene. Based on the available information, the health risks are likely to be similar to those for other opioid analgesics. In particular, the most serious acute health risk from isotonitazene is likely to be respiratory depression, which in overdose can lead to apnoea, respiratory arrest and death. This risk will be exacerbated by the use of other CNS depressants.

Acute toxicity

There is limited information on the acute toxicity of isotonitazene. Based on the available information, the health risks are likely to be similar to those for other opioid analgesics. These include sedation, bradycardia, hypothermia and respiratory depression.

While there is no information on the clinical features of poisoning caused by isotonitazene, they are likely to include miosis, a reduced level of consciousness or unconsciousness, and respiratory depression. Similar to other opioid analgesics, the most serious acute risk arising from the use of isotonitazene is likely to be respiratory depression, which in overdose can lead to apnoea, respiratory arrest and death. Although there is no reported clinical experience with poisonings caused by isotonitazene, based on the pharmacological properties of isotonitazene, naloxone is expected to work as an antidote. Owing to the short half-life of naloxone, it is possible that larger than normal doses and repeated doses may be required to manage the poisoning in some cases; longer periods of observation may also be required.

Similarly to other opioid analgesics, the use of isotonitazene with other CNS depressants, including other opioids, sedatives/hypnotics, alcohol, pregabalin, gabapentin, tranquillisers and sedating anti-histamines, is likely to produce additive depressant effects, which can increase the risk of life-threatening respiratory depression.

Acute poisonings

No cases of acute poisoning involving isotonitazene have been reported to the EMCDDA or published in the scientific literature.

Deaths

A total of 23 deaths associated with isotonitazene have been reported by four countries to date: Germany (1 case), the United Kingdom (1 case), Canada (3 cases) and the United States (18 cases). The role of isotonitazene in these deaths is unknown.

Deaths reported in Europe

A total of two deaths involving isotonitazene have been reported to the EMCDDA, by Germany and the United Kingdom. No further information is currently available for the death reported by Germany. The United Kingdom reported a death with confirmed exposure to isotonitazene that occurred in 2019. Alongside isotonitazene, butyrylfentanyl, despropionyl fentanyl (4-ANPP) and despropionyl fluorofentanyl were also identified in post-mortem biological samples; all the substances were estimated to be present in below ng/ml concentrations. No further details on the case are currently available.

Deaths reported elsewhere

In addition to the cases in Europe, deaths in which isotonitazene was identified in post-mortem samples have been reported in Canada (3 cases) and the United States (18 cases).

In Canada, isotonitazene was identified in three deaths during 2019. The deaths occurred in Alberta in March, September and October 2019. No further details are available.

In the United States, isotonitazene was identified in 18 deaths that occurred between August 2019 and January 2020; all cases were medico-legal death investigations. Two thirds of individuals were male ($n = 12$; 67 %). The mean age was 41 ± 12 years (median 41 years) and ranged from 24 to 66 years.

The mean concentration of isotonitazene in blood samples ($n=18$) was 2.2 ± 2.1 ng/mL (median: 1.75 ng/mL, range 0.4-9.5 ng/mL) (central or peripheral blood). The lowest concentration of isotonitazene in blood was encountered in two cases in which no other opioids were present. The mean concentration of isotonitazene in urine samples ($n = 6$) was 2.4 ± 1.4 ng/mL (median 2.7 ng/mL, range 0.6-4.0 ng/mL). The concentration of isotonitazene in a single sample of vitreous fluid was 0.1 ng/mL. It is important to note that quantitative results for isotonitazene were at the sub-nanogram level, which highlights a need for increased analytical scrutiny when testing for the substance.

Based on information from the death investigations ($n = 8$) and forensic toxicology results ($n = 18$), at least some of the individuals were high-risk drug users and included people who had a history of injecting established illicit opioids, such as heroin and fentanyl. Isotonitazene was identified along with one or more other psychoactive substances (controlled drugs and NPS) in all the deaths, which suggests that polydrug use was common in these individuals. Other findings in blood were as follows.

- In addition to isotonitazene, one or more other types of opioids were identified in 10 (56 %) of the cases; these included fentanyl ($n = 6$), morphine ($n = 3$) (including 6-monoacetylmorphine in one case), tramadol ($n = 4$), piperidylthiambutene ($n = 2$) and U-47700 ($n = 1$). Isotonitazene was the only opioid identified in the remaining eight cases.
- Benzodiazepines were identified in 16 (89 %) of the cases; most of the identifications related to flualprazolam ($n = 11$) or etizolam ($n = 8$).
- Stimulants were identified in six (33 %) of the cases: cocaine was identified in 4 (22 %) cases and amphetamine and methamphetamine were identified in two (11 %) cases (in one of these cases MDMA and MDA were also identified).
- Naloxone (an antidote used in opioid overdose) was identified in six (33 %) of the cases. This includes two cases in which isotonitazene was the only opioid identified.

Physical, mental and behavioural effects

The physical effects of isotonitazene have not been studied. Based on the pharmacological properties of the substance, as well as information from the study of the closely related substances etonitazene and metonitazene, it is likely that the effects of isotonitazene are similar to those commonly reported for other opioid analgesics, including miosis, analgesia, respiratory depression, constipation, bradycardia and hypothermia.

The mental and behavioural effects of isotonitazene have not been studied. Based on the pharmacological properties of the substance, as well as on information from the study of the closely related substances etonitazene and metonitazene, it is likely that the effects of isotonitazene are similar to those commonly reported for other opioid analgesics, including dizziness, drowsiness, mental confusion, incoordination, relaxation and euphoria; at higher doses, sedation and profound intoxication would be expected.

There have been no studies of the effects of isotonitazene on the ability to drive and operate machines. However, it is well established that opioid analgesics impair the mental and physical ability required to drive and operate machines. This effect is likely to extend to isotonitazene.

Chronic toxicity

There is no information on the chronic toxicity of isotonitazene.

Abuse liability and dependence-producing potential

The abuse liability and dependence-producing potential of isotonitazene have not been studied. However, etonitazene and metonitazene, both of which are closely related to isotonitazene, as well as clonitazene, have been studied to varying degrees in animals and, to some extent, in humans. Similar to other MOP receptor agonists, such as morphine, and fentanyl, these studies suggest that members of the 2-benzylbenzimidazole group of opioids, including isotonitazene, are likely to have an abuse liability and dependence-producing potential in humans. Research will be required in order to determine the degree of such effects.

Other relevant information

Accidental exposure, including occupational exposure, to isotonitazene — especially inhalation or ingestion — may pose a risk of poisoning to those who may come into contact with the substance.

This includes the family and friends of people who use isotonitazene, law enforcement, emergency personnel, medical and forensic laboratory personnel, as well as individuals in custodial settings and postal services. In addition to exercising extreme caution when handling materials suspected to contain isotonitazene, working environments and personnel should be equipped with appropriate protective equipment. The antidote naloxone should be readily available to personnel in sufficient quantities; training in resuscitation and naloxone administration should also be available.

Social risks

There is no specific information on the social risks posed by isotonitazene. There is no information on whether or not criminal groups are involved in the manufacture, trafficking and distribution of isotonitazene within Europe.

As isotonitazene acts as an opioid analgesic, it is possible that any such risks may have some similarities with those associated with the use of established illicit opioids, such as heroin or fentanyl and its derivatives. Such risks include negative impacts on social functioning and criminal activities, such as the involvement of organised crime in the manufacture, trafficking and distribution of the substance.

Extent and patterns of use, availability and potential for diffusion

There is limited information on the extent and patterns of use, availability and potential for diffusion of isotonitazene in Europe. As isotonitazene has been on the drug market for only a short period of time, no formal epidemiological studies have been conducted, which limits the understanding of the extent and patterns of use, as well as the potential for diffusion. In relation to this, the presence of isotonitazene on the drug market and its involvement in serious adverse events may be undetected in some areas because the substance is not routinely screened for in some forensic laboratories.

Isotonitazene has been available on the drug market in Europe since at least April 2019, when it was seized for the first time by police in Estonia. The most recent identification of isotonitazene reported to the EMCDDA was a seizure made by police in Latvia in January 2020. As of 1 May 2020, it has been identified in six Member States: Belgium, Estonia, Germany, Latvia, Sweden and the United Kingdom. These detections relate to police seizures reported by Estonia, Germany and Latvia, a customs seizure reported by Sweden, a collected sample reported by Belgium, and biological samples from a death case reported by the United Kingdom. While the seized quantities are relatively small, they should be seen within the context of the high potency of isotonitazene.

Based on information from a seizure reported by Sweden, it appears that at least some of the isotonitazene on the market in Europe has been supplied from China. Isotonitazene is sold online as a powder in wholesale and small amounts; it is also sold as ready-to-use nasal sprays. Based on limited information from police seizures reported by two Member States (Estonia and Latvia) it is presumed, but not confirmed, that isotonitazene has been sold on the illicit opioid market at street level and is being injected intravenously by people who use opioids. Overall, the demand for isotonitazene, and potential for diffusion, is unknown.

Isotonitazene may be deliberately sought after by some people, particularly those who self-experiment with a range of psychoactive substances (so-called 'psychonauts'); others, such as those who purchase it on street-level opioid markets, may be unaware that they are using a substance that presents an inherent risk to the individual. Although the drug situation is different from that in Europe, information from deaths with confirmed exposure to isotonitazene in the United States found that at

least some of the individuals were high-risk drug users and included people who had a history of injecting opioids such as heroin. In addition, isotonitazene was identified along with one or more other psychoactive substances (controlled drugs and NPS) in all the deaths, which suggests that polydrug use was common in these individuals. In particular, the use of other CNS depressants such as other opioids and benzodiazepine sedative/hypnotics was very common in these individuals.

Information on the dosage regimens used for isotonitazene is limited. The small number of anecdotal self-reported experiences on websites for people who use drugs suggest that, similar to other opioid analgesics, the dosage regimens used for isotonitazene can differ within and between individuals. It is not possible currently to discern typical dosage regimens. These also depend on the tolerance of the person who is using the substance, the use of other drugs and the desired effects. Furthermore, the purity, amount and/or composition of the substance ingested are not typically known by the people who are using the substance. In addition, the actual composition of the substance may differ over time and place. Nonetheless, a range of dosage regimens, including differing routes of administration (e.g. intravenous injection and nasal insufflation by spray), formulations (dosage forms) and the schedule of doses of isotonitazene — including the amount taken each time, time between doses and the duration of use — have been noted on websites for people who use drugs; polydrug use has also been noted as part of some of these self-reported experiences.

Commercial and industrial uses, the extent of such use and its use for scientific research and developmental purposes

Based on the available information reported to the EMCDDA from the EMA, it appears that isotonitazene is not an active substance in any medicinal product for human use or in any veterinary medicinal product in Europe. However, although unlikely, the use of isotonitazene as an active substance in medicinal products prepared extemporaneously or in investigational medicinal products cannot be excluded in some Member States.

There is currently no information suggesting that isotonitazene is used for legitimate purposes, including commercial and industrial uses, other than a scientific research or forensic application.

Other relevant information

Restrictive measures

International restrictive measures

At international level, isotonitazene is not controlled under the United Nations Single Convention on Narcotic Drugs, 1961, nor under the Convention on Psychotropic Substances of 1971. Isotonitazene has not been subject to assessment nor is it currently under assessment by the United Nations system.

National restrictive measures

Twenty-two Member States (Austria, Belgium, Bulgaria, Croatia, Cyprus, Czechia, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Luxembourg, Malta, the Netherlands, Portugal, Romania, Slovakia, Slovenia and Spain) reported that isotonitazene is not subject to restrictive

measures at national level. Turkey also reported that isotonitazene is not subject to restrictive measures at national level.

Isotonitazene is subject to restrictive measures in six Member States: in Estonia, Latvia, Poland and Sweden the substance is controlled under drug control legislation; in Lithuania it is controlled under medicines legislation; and in the United Kingdom it is controlled by NPS legislation. In addition, isotonitazene is controlled under medicines legislation in Norway.

It is unknown if isotonitazene is controlled in China, from where at least some of the substance on the European market appears to have been sourced.

COVID-19 pandemic

The impact of the ongoing COVID-19 pandemic on the manufacture, trafficking, distribution and use of isotonitazene is currently unknown. Based on previous experiences with disruptions to the illicit opioid markets in Europe and the United States, it is conceivable that, should there be a reduced availability of established illicit opioids such as heroin and fentanyl and its derivatives in Europe, then criminal groups, as well as people who use opioids, especially high-risk opioid users, may substitute for these substances a range of other substances, including 2-benzylbenzimidazole opioids such as isotonitazene. These changes may be geographically localised or broader; they may also be single 'one-off' events, or short-lived or longer-lasting changes. Similar to the recent experience with highly potent fentanyl derivatives, such as acryloylfentanyl and carfentanil, such changes to the drug market may increase the risk of life-threatening poisoning, as, currently, there is little to no experience with the use of isotonitazene (e.g. how to dose the substance and the effects it can cause); in some cases such substitution could manifest as outbreaks of poisoning. These risks will be especially high in the case of people who obtain isotonitazene from street-level illicit opioid markets and who are unlikely to be aware that they are using the substance.

Conclusion

Isotonitazene (*N,N*-diethyl-2-[[4-(1-methylethoxy)phenyl]methyl]-5-nitro-1*H*-benzimidazole-1-ethanamine) belongs to the 2-benzylbenzimidazole group of opioid analgesics. This group includes etonitazene and clonitazene, both of which are under international control. Although isotonitazene was first synthesised in the mid-1950s, no additional reports relating to the substance could be found until its identification on the illicit drug market in 2019.

The available information suggests that isotonitazene acts as a potent narcotic opioid analgesic. Similar to other opioid analgesics, the most serious acute risk arising from the use of isotonitazene is likely to be from respiratory depression, which can lead to apnoea, respiratory arrest and death. Naloxone is expected to work as an antidote to poisoning caused by isotonitazene.

Isotonitazene has been available in Europe since at least April 2019 and has been identified in six Member States (Belgium, Estonia, Germany, Latvia, Sweden and the United Kingdom). Law enforcement seizures have been reported by three Member States (Estonia, Latvia and Sweden). The most recent identification of isotonitazene reported to the EMCDDA was from a seizure made in January 2020.

As isotonitazene has emerged on the drug market in Europe only recently, it is important to note that its presence on the drug market and as the cause of serious adverse events may be undetected

because the substance is not routinely screened for in some laboratories. In addition, the ongoing COVID-19 pandemic may have reduced the capacity of early warning systems to detect and report events involving isotonitazene.

It appears that at least some of the isotonitazene on the market in Europe has been supplied from China. Isotonitazene is sold online as a powder in wholesale and small amounts; it is also sold as ready-to-use nasal sprays. In addition, isotonitazene is sold on the illicit drug market, including, it appears, on street-level opioid markets. Based on the limited information, it is presumed, but not confirmed, that isotonitazene is being injected intravenously by high-risk opioid users in some parts of Europe.

Isotonitazene can be administered in a range of ways, including orally, intranasally, by smoking or vaporising and by injection.

A total of two deaths involving isotonitazene have been reported to the EMCDDA by two Member States (Germany and the United Kingdom), although few additional details are currently available on these cases. Deaths have also been reported in Canada (3 cases) and the United States (18 cases). Of the cases in the United States, at least some of the individuals were high-risk drug users and included people who had a history of injecting illicit opioids such as heroin. Polydrug use, especially use of two or more CNS depressants (which increases the risk of life-threatening respiratory depression), was also common in these individuals.

There is no specific information on the social risks posed by isotonitazene. As isotonitazene acts as an opioid analgesic, it is possible that any such risks may have some similarities with those associated with the use of established illicit opioids, such as heroin or fentanyl and its derivatives.

There is no information on whether or not criminal groups are involved in the manufacture, trafficking and distribution of isotonitazene within Europe. The impact of the ongoing COVID-19 pandemic on the manufacture, trafficking, distribution and use of isotonitazene is also currently unknown. Based on previous experiences with disruptions to the illicit opioid markets, it is conceivable that, should the availability of established illicit opioids such as heroin and/or fentanyl and its derivatives be reduced in Europe, then criminal groups, as well as people who use opioids, especially high-risk opioid users, may substitute for these substances a range of other substances, including 2-benzylbenzimidazole opioids such as isotonitazene. Such changes may increase the risk of life-threatening poisoning, as, currently, there is little to no experience with the use of isotonitazene (such as doses and effects). These risks will be especially high in the case of people who obtain isotonitazene from street-level illicit opioids markets and who are unlikely to be aware that they are using the substance. The Committee notes that, both for isotonitazene, as well as for opioids in general, a high level of vigilance is needed to ensure early detection, reporting, assessment, and response measures to changes in the drug markets relating to the pandemic that may have a high impact on public health.

The Committee notes that while the available information on isotonitazene indicates that it is a potent opioid analgesic, overall there is limited information available on its pharmacology, toxicology, abuse liability and dependence-producing potential. Additional information may emerge with later studies but ideally would have been available in this assessment. The Committee notes that, to strengthen early warning, preparedness and response activities within the EU, such studies should be undertaken systematically prior to risk assessment.

There is no information on the actual chemical precursors or manufacturing methods used to synthesise the isotonitazene that has been identified on the drug market in Europe. Although the

synthetic routes reported in the scientific literature involve several steps, depending on precursor availability, they are straightforward and make use of common manufacturing equipment. The starting materials for either synthetic route are readily available. The multi-step production of isotonitazene requires a basic knowledge of and experience in synthetic organic chemistry.

Based on the available information, it appears that isotonitazene is not an active substance in any medicinal product for human use or in any veterinary medicinal product in Europe. However, although unlikely, the use of isotonitazene as an active substance in medicinal products prepared extemporaneously or in investigational medicinal products cannot be excluded in some Member States. There is currently no information suggesting that isotonitazene is used for legitimate purposes other than research or forensic applications.

Isotonitazene is not listed for control in the Single Convention on Narcotic Drugs, 1961, nor in the Convention on Psychotropic Substances, 1971. Isotonitazene is not currently under assessment by the United Nations system.

The available information would suggest that the consumption of isotonitazene causes harm to health associated with its acute toxicity and abuse liability or dependence-producing potential. This harm to health is considered life-threatening because it is likely to cause death. In addition, there is a potential for severe physical and mental impairment and a significant spread of diseases, including the transmission of blood-borne viruses. These effects, including dependence, are comparable to those of other opioid analgesics that are under international control, such as morphine, fentanyl and the closely related substance etonitazene.

Four Member States (Estonia, Latvia, Poland and Sweden) control isotonitazene under drug control legislation. Two Member States (Lithuania and the United Kingdom) and Norway control isotonitazene under other legislation.

As for any NPS, many of the questions related to isotonitazene that are posed by the lack of data on the risks to individual health, risks to public health and social risks could be answered through further research. Areas where additional information would be important include studies on epidemiology (frequency and patterns of use, including studies that examine the groups of people who use isotonitazene and risk behaviours); the market; chemical profiling; extended pharmacological profiling; metabolic pathways; behavioural effects; acute and chronic toxicity; the potential interaction between isotonitazene and other substances; the abuse liability and dependence-producing potential; and the public health and social risks associated with its use.

The Committee notes that a decision to control isotonitazene has the potential to bring with it both intended and unintended consequences. Potential intended consequences include reduced levels of availability and ultimately use. This may reduce the health and social risks and consequences arising from the use of isotonitazene. It is important to recognise that a potential unintended consequence of control may be the manufacture and availability of other substances, including the emergence of other compounds from the 2-benzylbenzimidazole group of opioids. The implementation of control measures may also lead to the criminalisation of those who continue to use this substance, with the possible attendant risks of socioeconomic stigmatisation and marginalisation.

Finally, the Committee notes that it is important to continue to collect accurate information on isotonitazene and to disseminate it to people who use the substance, as well as to practitioners, policymakers and decision-makers.

ANNEX 1

Technical report on *N,N*-diethyl-2-[[4-(1-methylethoxy)phenyl]methyl]-5-nitro-1*H*-benzimidazole-1-ethanamine (isotonitazene)

Purpose

The purpose of this technical report to provide an analyses of the available information on *N,N*-diethyl-2-[[4-(1-methylethoxy)phenyl]methyl]-5-nitro-1*H*-benzimidazole-1-ethanamine (commonly known as isotonitazene), an opioid analgesic that has recently emerged on the drug market in Europe, to support a risk assessment of the substance that has been requested by the European Commission in accordance with Article 5c of Regulation (EC) No 1920/2006 (as amended).

Parts of this report were prepared under an EMCDDA contract (ref. CT.20.SAS.0017.1.0).

Statement regarding the United Kingdom

The reference period for this technical report includes 2019 and 2020 (up to the time of writing). The United Kingdom left the European Union as of 1 February 2020. However, during the transitional period, the United Kingdom continues to participate in the European Union Early Warning System on new psychoactive substances. Unless stated otherwise, for the purpose of this report, the term 'Member States' shall include the United Kingdom.

Information sources

The information in this technical report is derived from:

- Information reported by the Member States, Turkey, and Norway to the EMCDDA and Europol in accordance with the requirements of Article 5a and Article 5b of Regulation (EC) No 1920/2006 (as amended).
- Information reported by the European Medicines Agency (EMA), the European Chemicals Agency (ECHA), the European Centre for Disease Prevention and Control (ECDC), and the European Food Safety Authority (EFSA) to the EMCDDA in accordance with the requirements of Article 5b of Regulation (EC) No 1920/2006 (as amended).
- A review of the benzimidazole opioids commissioned by the EMCDDA (CT.20.SAS.0017.1.0).
- Information collected by the EMCDDA through searches of open source information, including the scientific and medical literature, patents, official reports, grey literature, online drug discussion forums and related websites, and online vendors selling isotonitazene.

Search strategy

Literature searches used both chemical structure and textual queries in online databases; searches were conducted in March 2020. The retrieved publications were then scanned for additional relevant references (snowballing technique).

SciFinder® was searched by exact structure-based search. PubMed and Web of Science were searched for 'isotonitazene' and the IUPAC name of this compound stated in this document. The references were screened for relevance and included in the review where appropriate. Additional references were gathered from the sources mentioned in the collected papers.

Terminology and definitions

The terminology and definitions used in this technical report are based on those used for the operation of the EU Early Warning System on new psychoactive substances, including those related to relevant internal EMCDDA processes. They can be accessed at:

<http://www.emcdda.europa.eu/system/files/publications/12213/downloads/Guidance%20Note%201-%20Terminology%20and%20definitions.pdf>

Unless otherwise indicated, the terms and definitions are for operational use only and do not have legal meaning. They may differ from those used in other settings and by other organisations (EMCDDA, 2019).

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1. Summary

During the mid-1950s, attempts to develop better and safer opioid analgesics led to the discovery of a series of 2-benzylbenzimidazole compounds with levels of analgesic potency several orders of magnitude higher than that of morphine. This group of structurally distinct opioid analgesics includes isotonitazene, the subject of this technical report, as well as etonitazene and metonitazene which are closely related homologues, and clonitazene. Both etonitazene and clonitazene are controlled under the United Nations Single Convention on Narcotic Drugs of 1961 (ECAPD, 1961; UNODC, 2019a)

Although isotonitazene was first synthesised in the mid-1950s (Hoffmann et al., 1959; Hoffmann et al. 1960; Hunger et al., 1960b), no additional reports related to the substance could be found until its identification on the illicit drug market in 2019 (Blanckaert et al., 2020; EMCDDA, 2020a; Ujváry, 2020).

In Europe, isotonitazene is monitored as a new psychoactive substance by the EMCDDA in accordance with Council Framework Decision 2004/757/JHA (as amended) and Regulation (EC) No 1920/2006 (as amended) (EMCDDA, 2020a). The substance has been available on the drug market in Europe since at least April 2019. As isotonitazene has only recently emerged on the drug market, there is limited information on the substance. In particular, formal epidemiological studies have not been conducted, which limits understanding of the frequency and patterns of use of isotonitazene. As of 6 May 2020, isotonitazene is the only substance from the 2-benzylbenzimidazole series of opioid analgesics to be notified to the EMCDDA ⁽²⁾.

As isotonitazene has only recently emerged on the drug market in Europe, it is important to note that its presence on the market and as the cause of serious adverse events (such as from acute poisonings presenting to hospital emergency rooms and medico-legal death investigations) may be undetected since the substance is not routinely screened for in some laboratories. An additional issue is that concentrations of isotonitazene in biological samples are typically low to sub-nanogram per millilitre which highlights a need for increased analytical sensitivity when testing for the substance. It is also important to note that, in some settings, the ongoing COVID-19 pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (ECDC, 2020; EMCDDA, 2020b; WHO, 2020) may have reduced the capacity of early warning systems, including forensic science and toxicology laboratories, to detect and report events involving isotonitazene.

As of 28 March 2020, the EMCDDA has received reports that isotonitazene has been identified in six Member States: Belgium, Estonia, Germany, Latvia, Sweden, and the United Kingdom. These detections relate to police seizures reported by Estonia, Germany, and Latvia; a customs seizure reported by Sweden; a collected sample reported by Belgium; and biological samples from a death case reported by the United Kingdom. While the detected quantities are relatively small, they should be seen within the context of the possible high potency of isotonitazene. The most recent identification of isotonitazene reported to the EMCDDA is from a seizure made by police in Latvia in January 2020.

Although isotonitazene has not been formally studied in humans, a study published in 2019 has demonstrated that it is a highly potent, full mu-opioid receptor (MOP) agonist *in vitro* (Blanckaert et al., 2020), while an animal study published in 1960 has demonstrated that it has potent morphine-like

⁽²⁾ In accordance with Council Framework Decision 2004/757/JHA (as amended) and Regulation (EC) No 1920/2006 (as amended).

centrally-mediated analgesic effects (Hunger et al., 1960b). Due to its lipophilicity, isotonitazene is expected to be rapidly absorbed and readily cross the blood-brain barrier. Taken together, this information strongly suggests that isotonitazene will act as an opioid analgesic in humans. The major pharmacological effects of opioid analgesics are due to their activation of opioid receptors, and, in particular, the mu-opioid receptor. Besides their analgesic properties, a notable feature associated with opioid analgesics is that they cause dose-dependent respiratory depression (slowing down of breathing), in which overdose can be life-threatening. Other additional pharmacological effects include miosis, sedation, bradycardia, hypothermia, constipation, physical dependence, and changes in mood such as euphoria (Herz, 1993; Kieffer, 1999; Pasternak and Pan, 2013; Pattinson, 2008; Romberg et al., 2003).

Based on the available pharmacological information, and similar to other opioid analgesics, the most serious acute risk from the use of isotonitazene in humans is likely to be from respiratory depression, which can lead to apnoea, respiratory arrest, and death (Pattinson, 2008; Romberg et al., 2003; White and Irvine, 1999). Compounding this risk is that isotonitazene is the first of the 2-benzylbenzimidazole opioids to be identified on the drug market in recent years, and users have no experience with this group of opioids (such as how to dose the substance and the effects it causes) which may increase the risk of accidental overdose and cause life-threatening poisoning. This risk will be especially high if users are unaware that they are using isotonitazene, which may be the case when it is sold at street-level on the illicit opioid market. While clinical experience in treating poisonings caused by isotonitazene have not been reported, based on the pharmacological profile of the substance, naloxone is expected to work as an antidote and reverse respiratory depression (Boyer, 2012; Kim and Nelson, 2015). There is no information on the chronic health effects of isotonitazene. Similar to other opioids, the chronic health risks might share some similarities to those seen with established illicit opioids, such as heroin and fentanyl; this may include dependence.

In Europe, a total of two deaths involving isotonitazene have been reported to the EMCDDA by Germany and the United Kingdom; few additional details are currently available on these cases. Deaths have also been reported in Canada (3 cases) (Toxicovigilance Canada, 2019) and the United States (18 cases) (Krotulski et al., 2020). In all cases, the role of isotonitazene in the deaths is unknown. In the deaths reported from the United States, at least some of the individuals were high risk drug users and included people who had a history of injecting opioids such as heroin. Polydrug use, especially use of two or more central nervous system (CNS) depressants (which increases the risk of life-threatening respiratory depression (US FDA, 2016)) was also common in these individuals (Krotulski et al., 2020).

There is limited information on the manufacture, trafficking, distribution, and use of isotonitazene in Europe. It appears that at least some of the isotonitazene on the market has been produced by chemical companies based in China. Although the size and scale of the operations are unknown, isotonitazene is sold online as a powder in wholesale and small amounts; it is also sold as ready-to-use nasal sprays. Isotonitazene may also have been sold on the illicit opioid market at street-level in at least two Member States in Europe (Estonia and Latvia). In what appears to be a small number of cases, isotonitazene may be deliberately sought after by some users (such as people who self-experiment with psychoactive substances ('psychonauts')); others, such as those that purchase it on street-level illicit opioids markets, may be unaware that they are using the substance which presents an inherent risk to the individuals.

There is no information on whether or not criminal groups are involved in the manufacture, trafficking, and distribution of isotonitazene within Europe (EMCDDA, 2020a). The impact of the ongoing COVID-19 pandemic (ECDD, 2020; EMCDDA, 2020b; WHO, 2020) on the manufacture, trafficking,

distribution, and use of isotonitazene is also currently unknown. Based on previous experiences with disruptions to the illicit opioid markets (Ciccarone, 2019; EMCDDA, 2011; EMCDDA, 2012; Mars et al., 2019), it is conceivable, that, should the availability of established illicit opioids, such as heroin and/or fentanyl and its derivatives, be reduced in Europe, then criminal groups, as well as people who use opioids, especially high risk opioid users, may substitute these substances for a range of other substances, including 2-benzylbenzimidazole opioids such as isotonitazene. These changes may be geographically localised or broader, they may also be single 'one off' events, or short-lived, or longer lasting changes. Similar to the recent experience with highly potent fentanyl derivatives, such as acrylylfentanyl and carfentanil, such changes to the drug market may increase the risk of life-threatening poisoning, as, currently, there is little to no experience with the use of isotonitazene (such as doses and effects); in some cases such substitution could manifest as outbreaks of poisoning (EMCDDA, 2018; Evans-Brown and Sedefov, 2018; Ujváry et al., 2017). These risks will be especially high in the case of people who obtain isotonitazene from street-level illicit opioids markets who are unlikely to be aware that they are using the substance.

There is no information on the social harms that may be caused by isotonitazene. Despite this, it is likely that some of the risks are similar to those associated with the use of established illicit opioids, such as heroin and fentanyl.

Based on the available information, it appears that isotonitazene is not an active substance in a medicinal product for human use or in a veterinary medicinal product in Europe. However, although unlikely, the use of isotonitazene as an active substance in medicinal products prepared extemporaneously or in investigational medicinal products cannot be excluded in some Member States (EMCDDA, 2020a). There is currently no information that suggests isotonitazene is used for legitimate purposes other than research or forensic application (EMCDDA, 2020a).

Isotonitazene is not controlled under the United Nations Single Convention on Narcotic Drugs, 1961, as amended by the 1972 Protocol, nor the Convention on Psychotropic Substances of 1971 ('United Nations system') (UNODC, 2019a; UNODC, 2019b). Isotonitazene has not been subject to assessment nor is it currently under assessment by the United Nations system (EMCDDA, 2020a). In Europe, isotonitazene is subject to restrictive measures in six Member States: in Estonia, Latvia, Poland, and Sweden the substance is controlled under drug control legislation; in Lithuania it is controlled under medicines legislation; while in the United Kingdom it is controlled by new psychoactive substance legislation. In addition, isotonitazene is controlled under medicines legislation in Norway. It is unknown if isotonitazene is controlled in China, where at least some of the substance on the European market has been sourced from (EMCDDA, 2020a).

2. Chemical and physical properties, methods and the precursors used for manufacture or extraction

2.1 Background

During the mid-1950s, attempts to develop better and safer opioid analgesics led to the discovery of a series of 2-benzylbenzimidazole compounds with levels of analgesic potency several orders of magnitude higher than morphine. This group of structurally distinct analgesics was invented by the pharmaceutical research laboratories of the Swiss chemical company CIBA Aktiengesellschaft (Hunger et al., 1957; Gross and Turrian, 1957; Hunger et al., 1960a,b). Though this particular research effort did not yield any marketed analgesic medicine, the bold departure from the morphine structure demonstrated that high levels of opioid activity could be achieved by substances structurally

less complex than morphine and by substances bearing no structural resemblance to the phenanthrene skeleton of morphine. This new series of compounds included *N,N*-diethyl-2-[[4-(1-methylethoxy)phenyl]methyl]-5-nitro-1*H*-benzimidazole-1-ethanamine (isotonitazene), the subject of this technical report, and the closely related homologues, etonitazene ⁽³⁾ and metonitazene ⁽⁴⁾, as well as clonitazene ⁽⁵⁾. Etonitazene and clonitazene are the most intensively studied compounds from the series (Ujváry, 2020). Shortly after their invention, and based on a notification by the United States Government, both etonitazene and clonitazene were controlled under the original Schedule I list of the United Nations Single Convention on Narcotic Drugs of 1961 because of their ability to produce morphine-like effects, to suppress abstinence phenomena of a known morphine addiction, as well as to sustain morphine addiction (ECAPD, 1961; Ujváry, 2020).

Although this series of compounds was highlighted by the chemist Alexander T. Shulgin more than 40 years ago as a 'fertile field for the search for heroin substitutes that can be domestically synthesized and are potent at levels that would encourage illicit investigation' (Shulgin, 1975), until 2019, when isotonitazene was first identified on the illicit drug market, only etonitazene had been sporadically encountered on the drug market: first in a 'brownish looking powder' in Milan, Italy, in 1966 (Branderberger, 1974), then in Germany in 1987 [cited by Sorokin et al., 1999], in Russia in 1998 and 1999 (Sorokin, 1999; Sorokin et al., 1999), and, finally, in the United States in 2003 (Reavy, 2003; Morris, 2009).

Although isotonitazene was one of 2-benzylbenzimidazole compounds reported in patents and in the original study on this group of substances (Hoffmann et al., 1959; Hoffmann et al. 1960; Hunger et al., 1960b), no additional reports related to isotonitazene could be found until its identification on the illicit drug market in 2019 (Blanckaert et al., 2020; EMCDDA, 2020a; Ujváry, 2020). As of 6 May 2020, isotonitazene is the only substance from the 2-benzylbenzimidazole series of opioid analgesics to be identified on the European drug market and notified to the EMCDDA through the European Union Early Warning System on new psychoactive substances ⁽⁶⁾.

2.2 Names and chemical structure

Isotonitazene belongs to the 2-benzylbenzimidazole group of opioid analgesics. In particular, it is a 5-nitro-2-benzylbenzimidazole. This group also includes the closely related homologues, etonitazene and metonitazene, as well as clonitazene.

Isotonitazene differs from etonitazene and metonitazene in the substitution at the *para*-position of the benzyl moiety, which is an isopropoxy group in isotonitazene, an ethoxy group in etonitazene, a methoxy group in metonitazene. Isotonitazene differs from clonitazene by replacement of the chloro

⁽³⁾ IUPAC name: *N,N*-diethyl-2-[[4-ethoxyphenyl]methyl]-5-nitro-1*H*-benzimidazole-1-ethanamine

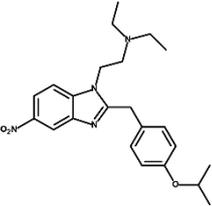
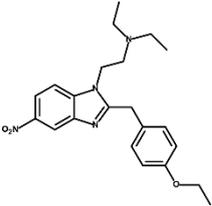
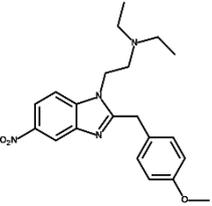
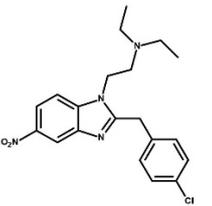
⁽⁴⁾ IUPAC name: *N,N*-diethyl-2-[[4-methoxyphenyl]methyl]-5-nitro-1*H*-benzimidazole-1-ethanamine

⁽⁵⁾ IUPAC name: *N,N*-diethyl-2-[[4-chlorophenyl]methyl]-5-nitro-1*H*-benzimidazole-1-ethanamine

⁽⁶⁾ In accordance with Council Framework Decision 2004/757/JHA (as amended) and Regulation (EC) No 1920/2006 (as amended).

halogen atom with the ethereal isopropoxy group. The chemical structure and molecular properties of these compounds are provided in Figure 1 (7).

FIGURE 1
Chemical structure and molecular properties of isotonitazene, etonitazene, metonitazene, and clonitazene.

Isotonitazene	Etonitazene	Metonitazene	Clonitazene
			
Molecular formula: <chem>C23H30N4O3</chem>	Molecular formula: <chem>C22H28N4O3</chem>	Molecular formula: <chem>C21H26N4O3</chem>	Molecular formula: <chem>C20H23ClN4O2</chem>
Molecular weight: 410.51	Molecular weight: 396.48	Molecular weight: 382.46	Molecular weight: 386.88
Monoisotopic mass: 410.2318	Monoisotopic mass: 396.2161	Monoisotopic mass: 382.2005	Monoisotopic mass: 386.1510
logP: 4.85	logP: 4.85	logP: 4.086	logP: 4.85
miLogP: 5.08	miLogP: 4.71	miLogP: 4.34	miLogP: 4.96
TPSA: 76.11 Å ²	TPSA: 76.11 Å ²	TPSA: 76.11 Å ²	TPSA: 66.88 Å ²

The octanol/water distribution coefficients logP (8) or miLogP were calculated by Molinspiration property engine v2018.10 (9) and StarDrop® Version 6.6 software, (10) respectively. The topological polar surface area (TPSA) (11) was calculated by StarDrop® Version 6.6 software.

Common name:
Isotonitazene

(7) Another closely related substance is the n-propoxy isomer of isotonitazene, known as pronitazene (*N,N*-diethyl-2-[(4-propoxyphenyl)methyl]-5-nitro-1*H*-benzimidazole-1-ethanamine). Pronitazene differs from isotonitazene in the substitution at the para-position of the benzyl moiety, which is an isopropoxy group in isotonitazene and a propoxy group in pronitazene. Information of the pharmacology of pronitazene can be found in Hunger et al., (1960b) (compound XLIII in Table I); briefly pronitazene was around 200 times more potent than morphine as an antinociceptive drug in mice, and, after etonitazene and isotonitazene, was the third most potent substance in the series (Hunger et al., 1960b).

As discussed in section 2.5, GC-MS analysis of isotonitazene and pronitazene will result in very similar mass spectrometry fragmentation patterns. The ability to distinguish between both isomers requires the use of analytical reference standards, access to reference spectra for both substances, or additional analytical methods.

(8) LogP is the logarithm of the partition of a substance between an apolar organic solvent, typically n-octanol, and water. It is used as a measure of lipophilicity of a substance. For fentanyl, calculated logP values are 3.89 (StarDrop) and 3.79 (Molinspiration), while the measured logP = 4.05 (Hansch et al., 2005).

(9) <https://www.molinspiration.com/cgi-bin/properties>

(10) Optibrium Ltd, Cambridge, UK.

(11) Topological polar surface area (TPSA) is a calculated measure the surface area occupied by nitrogen and oxygen atoms and the polar hydrogen atoms attached to them. For drugs acting on the central nervous system a TPSA is usually <90 Å². For fentanyl, the calculated TPSA = 23.6 Å².

Systematic (IUPAC) name:

N,N-diethyl-2-[[4-(1-methylethoxy)phenyl]methyl]-5-nitro-1*H*-benzimidazole-1-ethanamine

Other chemical names:

N,N-diethyl-2-[2-({4-[(propan-2-yl)oxy]phenyl}methyl)-5-nitro-1*H*-benzimidazol-1-yl]ethan-1-amine

N,N-diethyl-2-(2-(4-isopropoxybenzyl)-5-nitro-1*H*-benzo[d]imidazol-1-yl)ethan-1-amine

N,N-diethyl-2-[2-(4-isopropoxybenzyl)-5-nitro-1*H*-benzimidazol-1-yl]ethanamine

N,N-diethyl-2-[2-[(4-isopropoxyphenyl)methyl]-5-nitro-benzimidazol-1-yl]ethanamine

N,N-diethyl-2-[[4-(1-methylethoxy)phenyl]methyl]-5-nitro-1*H*-benzimidazole-1-ethanamine

N,N-diethyl-2-[5-nitro-2-[(4-propan-2-yloxyphenyl)methyl]benzimidazol-1-yl]ethanamine

1-[2-(diethylamino)ethyl]-2-(*p*-isopropoxybenzyl)-5-nitrobenzimidazole

Street names:

'iso', 'Toni'

Chemical Abstracts Service (CAS) registry numbers:

14188-81-9 free base

119276-00-5 hydrochloride salt

IUPAC International Chemical Identifier Key (InChI Key):

OIOQREYBGDAYGT-UHFFFAOYSA-N

Simplified Molecular-Input Line-Entry System (SMILES):

CCN(CC)CCN1C(CC2=CC=C(OC(C)C)C=C2)=NC3=CC([N+]([O-])=O)=CC=C31

2.3 Physical properties

Both the free base and salts of isotonitazene are solids.

The measured melting point for isotonitazene hydrochloride salt is 172–173 °C (Hoffmann et al., 1960; Hunger et al., 1960b).

Isotonitazene is lipophilic (calculated logP = 4.85).

Isotonitazene, as both the freebase and the hydrochloride salt, is soluble in methanol (NPS Discovery, 2019; Blanckaert et al., 2020) and in dimethyl sulfoxide (DMSO) (Blanckaert et al., 2020). Although no experimental data are available, the salts of isotonitazene, similar to etonitazene, are expected to be sufficiently water-soluble for injectable administration of effective doses.

To date, seizures and collected samples containing isotonitazene reported to the EMCDDA have been in brown, yellow, and white powders. In addition, isotonitazene has also been identified in liquid form. Identifications of isotonitazene reported to the EMCDDA include both the free base and the hydrochloride salt.

No studies have examined the stability and reactivity of isotonitazene. As a base, the substance readily forms salts with inorganic or organic acids. Chemical reducing agents are expected to convert the nitro group into an amino group. For metabolic conversions, see Section 4.4 on pharmacokinetics.

2.4 Methods and chemical precursors used for the manufacture or extraction

There is no information on the chemical precursors or manufacturing methods used to synthesise the isotonitazene which has been identified on the drug market in Europe.

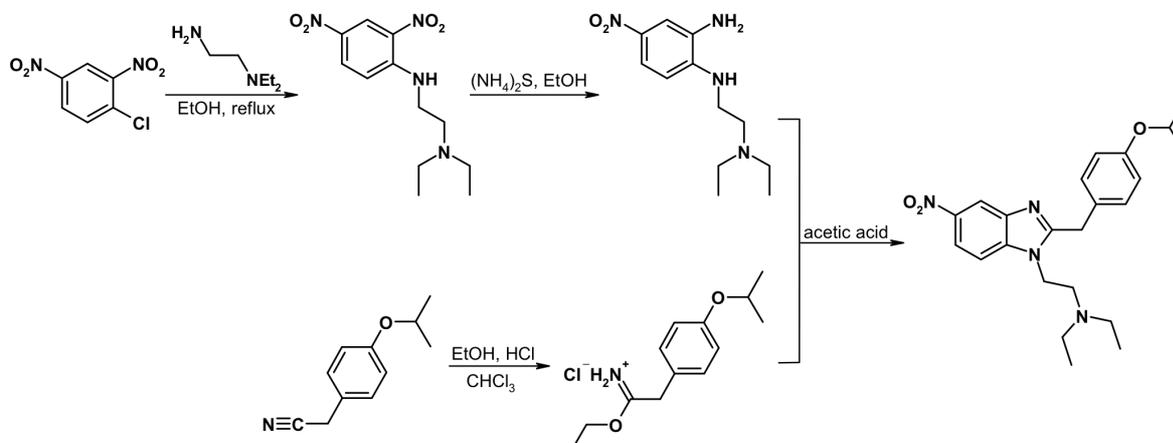
Several routes have been developed for the synthesis of etonitazene-related 2-benzylbenzimidazoles (Hunger et al., 1957; Hunger et al., 1960a). The synthesis of isotonitazene is specifically described in a patent and is outlined in Figure 2 (Hoffmann et al., 1960).

The activated chloro atom in the readily available 1-chloro-2,4-dinitrobenzene is easily displaced by 2-diethylaminoethylamine. Regioselective reduction of the nitro function adjacent to the alkylamino moiety of the resultant 2,4-dinitroaniline derivative is accomplished using ammonium sulfide. Condensation of the obtained *ortho*-phenylenediamine species ⁽¹²⁾ with the imidate of 4-isopropoxyphenylacetic acid, obtained from the corresponding cyanide, affords the final product, that is isotonitazene. Purification is accomplished by base-acid extraction followed by conversion of the free base into its hydrochloride salt.

Although there is no information on the actual method used for the manufacture of isotonitazene that has been identified on the drug market, one possible approach may follow the improved method as described for etonitazene by Carroll et al. (1975). The authors describe the method as simple, producing high yields, which can be adapted to both large scale preparations and for the preparations of other 2-benzylbenzimidazole opioids (Carroll et al., 1975). Alternatively, alkylation by isopropyl bromide of a phenolic species ('desethyletonitazene'), which was reported to be a versatile precursor for other homologues (Hoffmann et al., 1959; Hoffmann et al., 1960), may also be used to produce isotonitazene.

⁽¹²⁾ IUPAC name: N1-[2-(diethylaminoethyl)-4-nitrobenzene-1,2-diamine

FIGURE 2
Synthesis of isotonitazene (Hoffmann et al., 1960).



Analytically confirmed detections of the closely related homologue etonitazene on the illicit drug market in Moscow, Russia, were reported in 1998 and 1999 (Sorokin, 1999; Sorokin et al., 1999b). Information from one of these cases noted that the substance had been synthesised in Russia, using a modification of a method published by Hunger et al. (1960b). These reports also noted the identification of etonitazene on the illicit drug market in Germany in 1987, which also apparently used the synthetic route described earlier (Hunger et al., 1960b).

Recently, a ‘one-pot’, three component synthesis producing 2-benzylbenzimidazole opioids at high yield has also been reported (Kim et al., 2011).

2.5 Methods for identification and analysis

Methods documented in the literature for the identification and analysis of isotonitazene in physical samples include: gas chromatography–mass spectrometry (GC-MS); Fourier transform infrared spectroscopy (FTIR), ^1H and ^{13}C nuclear magnetic resonance spectroscopy (NMR), Raman spectroscopy and ultraviolet spectroscopy; and high-performance liquid chromatography (HPLC) (ADEBAR, 2020; Blanckaert et al., 2020; Cayman Chemical, 2020a; Krotulski et al., 2020; NPS Discovery, 2019) (Table 1). Isotonitazene is available as analytical reference material (Cayman Chemical, 2020a).

TABLE 1

Analytical methods used for the characterisation of isotonitazene in physical samples (Ujváry, 2020).

Analytical method	Reference
High-performance liquid chromatography	NPSDiscovery, 2019 ADEBAR, 2020 Blanckaert <i>et al.</i> , 2020
Ultraviolet spectroscopy	Blanckaert <i>et al.</i> , 2020 Cayman Chemical, 2020a
Infrared spectroscopy	ADEBAR, 2020 Blanckaert <i>et al.</i> , 2020
Raman spectroscopy	ADEBAR, 2020
¹ H NMR spectroscopy	ADEBAR, 2020 Blanckaert <i>et al.</i> , 2020
¹³ C NMR spectroscopy	ADEBAR, 2020 Blanckaert <i>et al.</i> , 2020
Mass spectrometry	NPSDiscovery, 2019 ADEBAR, 2020 Blanckaert <i>et al.</i> , 2020 Cayman Chemical, 2020a

Methods have also been documented in the literature for the identification of isotonitazene in biological samples, which include HPLC and liquid chromatography with tandem mass spectrometry (LC-MS/MS) (Krotulski *et al.*, 2020). It is important to highlight the need for increased analytical sensitivity when testing for isotonitazene in biological samples following quantitative results from blood and urine samples which were in some cases in the sub-nanogram per millilitre range (Krotulski *et al.*, 2020).

It is important to note that GC-MS analysis of isotonitazene and the *n*-propoxy isomer of isotonitazene, protonitazene (¹³), will result in very similar mass spectrometry fragmentation patterns. The ability to distinguish between both isomers requires the use of analytical reference standards, access to reference spectra for both substances, or additional analytical methods. Protonitazene is available as analytical reference material (Cayman Chemical, 2020b).

2.6 Dosage regimens

Information on the dosage regimens (¹⁴) used for isotonitazene is limited. Isotonitazene (¹⁵) can be administered orally as a powder, as tablets, or as a solution; it can also be administered intranasally

(¹³) IUPAC name for protonitazene: *N,N*-diethyl-2-[(4-propoxyphenyl)methyl]-5-nitro-1*H*-benzimidazole-1-ethanamine

(¹⁴) Dosage regimen: is information on the formulation (dosage form), route of exposure, as well as the schedule of doses of a new psychoactive substance, including the amount taken each time, time between doses, and the duration of use.

(¹⁵) It may be assumed that seizures contain isotonitazene in a salt form, which is water-soluble. There is, however, no information on the aqueous solubility of the salt.

or sublingually via spray or snorted (insufflated); inhaled by vaporising e-liquid solutions ('vaping'); inhaled by smoking or vaporising the 'free base'; and injected.

Given the illicit nature of the trade in isotonitazene, the composition of physical samples (law enforcement seizures and collected samples) is likely to vary over time and place, as well as based on the specific location in the drug supply chain in which the sample was obtained from (for example, from the manufacturer, wholesaler, retailer, or at street-level illicit opioid markets). In Europe, isotonitazene has been seized by law enforcement predominately in powder form; in two cases it has also been seized as a liquid. Information on the amount of isotonitazene present in seized powders (chemical purity) has not been reported, however information from police seizures in Estonia noted the presence of 'common sugars' in at least some of the samples (not specified further); while one seizure made by police in Latvia also contained fentanyl. Results from the analysis of a collected sample of isotonitazene in powder form that was purchased from an online vendor noted that isotonitazene was the only substance present in the sample and no impurities were identified. The study concluded that the sample was the hydrochloride salt of the substance in high purity (Section 5.3) (Blanckaert et al., 2020). In Canada, isotonitazene has been identified both in powder form as well as in falsified (fake) opioid analgesic medicines (DSPM, 2020). In the latter case, this includes fake Dilaudid® tablets (hydromorphone hydrochloride) that were seized in February 2020 (Halifax Police, 2020).

Based on the limited information reported to the EMCDDA from police seizures, it is presumed, but not confirmed, that isotonitazene is being injected intravenously by high risk opioid users in some parts of Europe. Although the drug situation is different from Europe, information from a case series of 18 deaths with confirmed exposure to isotonitazene in the United States noted that at least some of the individuals were high risk drug users and included people who had a history of injecting opioids such as heroin. In addition, isotonitazene was identified along with one or more other psychoactive substances (controlled drugs and new psychoactive substances) in all the deaths, which suggests that polydrug use was common in these individuals (Krotulski et al., 2020) (Section 6.2.2).

While formal epidemiological studies have not been performed, the small number of anecdotal self-reported experiences on user websites suggest, that, similar to other opioid analgesics, the dosage regimens used for isotonitazene can differ within and between individuals. It is not possible to currently discern typically dosage regimens. These also depend on the tolerance of the user, the use of other drugs, and the desired effects. Furthermore, the purity, amount and/or composition of the substance ingested are not typically known by the user. In addition, the actual composition of the substance may differ over time and place. Nonetheless, a range of dosage regimens, including differing routes of administration (such as intravenous injection and nasal insufflation by spray), formulations (dosage forms), as well as the schedule of doses of isotonitazene, including the amount taken each time, time between doses, and the duration of use, have been noted on user websites; polydrug use has also been noted as part of some of these self-reported experiences.

3. Legitimate use

3.1 Summary

Based on the available information, it appears that isotonitazene is not an active substance in a medicinal product for human use or in a veterinary medicinal product in Europe. However, although highly unlikely, the use of isotonitazene as an active substance in medicinal products prepared extemporaneously or in investigational medicinal products cannot be excluded in some Member

States (EMCDDA, 2020a). There is currently no information that suggests isotonitazene is used for legitimate purposes other than research or forensic application.

3.2 Medical use

Based on information from the European Medicines Agency for the initial report (EMCDDA, 2020a), it appears that isotonitazene is not an active substance in:

- a medicinal product for human use or in a veterinary medicinal product that has obtained a marketing authorisation in accordance with Directive 2001/83/ EC of the European Parliament and of the Council, Directive 2001/82/EC of the European Parliament and of the Council or Regulation (EC) No 726/2004 of the European Parliament and of the Council;
- a medicinal product for human use or in a veterinary medicinal product that is the subject of an application for a marketing authorisation;
- a medicinal product for human use or in a veterinary medicinal product whose marketing authorisation has been suspended by the competent authority.

In addition, it appears that isotonitazene is not an active substance in the following, although the information, especially in relation to use in extemporaneously prepared products, is unknown in some cases:

- an unauthorised medicinal product for human use in accordance with Article 5 of Directive 2001/83/ EC or in a veterinary medicinal product prepared extemporaneously by a person authorised to do so under national law in accordance with point (c) of Article 10(1) of Directive 2001/82/EC;
- an investigational medicinal product as defined in point (d) of Article 2 of Directive 2001/20/EC of the European Parliament and of the Council.

3.3 Industrial, commercial, and scientific use

Isotonitazene is used as an analytical reference material in clinical and forensic case work as well as scientific research. There is currently no information that suggests isotonitazene is used for other legitimate purposes.

As part of the initial report process, the European Chemical Agency (ECHA) and European Food Standard Authority (EFSA) reported to the EMCDDA that isotonitazene did not retrieve any results in their information systems (EMCDDA, 2020a).

4. Pharmacological and toxicological properties

4.1 Summary

There is limited information on the pharmacological properties of isotonitazene. Although isotonitazene has not been formally studied in humans, a study published in 2019 has demonstrated that it is a highly potent, full mu-opioid receptor (MOP) agonist *in vitro* (Blanckaert et al., 2020), while an animal study published in 1960 has demonstrated that it has potent morphine-like centrally-mediated analgesic effects (Hunger et al., 1960b). Due to its lipophilicity, isotonitazene is expected to be rapidly absorbed and readily cross the blood-brain barrier. Taken together, this information strongly suggests that isotonitazene will act as an opioid analgesic in humans. The major

pharmacological effects of such opioid analgesics, including their analgesic activity, are due to their activation of the MOP receptor. Besides their analgesic properties, a notable feature associated with opioid analgesics is that they cause dose-dependent respiratory depression, in which overdose can be life-threatening. Other additional pharmacological effects include miosis, sedation, bradycardia, hypothermia, constipation, physical dependence, and changes in mood such as euphoria (Herz, 1993; Kieffer, 1999; Pasternak and Pan, 2013; Pattinson, 2008; Romberg et al., 2003).

Although not formally studied, the psychological and behavioural effects of isotonitazene are likely to share some similarities with those commonly reported for other opioid analgesics, including: dizziness, drowsiness, mental confusion, incoordination, relaxation, and euphoria; at higher doses, sedation and profound intoxication would be expected.

The toxicological properties of isotonitazene have not been studied. Despite the lack of experimental data, observations that antinociceptive potency in general correlates with acute toxicity within the 2-benzylbenzimidazole group of opioids, including for etonitazene or metonitazene, that are both closely related homologues of isotonitazene, suggest that the acute toxicity of isotonitazene, at least in animal models, may be much greater than morphine and similar to that estimated for etonitazene or metonitazene.

Based on the available information, and, similar to other opioid analgesics, the most serious acute health risk from isotonitazene is likely to be life-threatening respiratory depression (Pattinson, 2008; Romberg et al., 2003; White and Irvine, 1999). The use of isotonitazene with other central nervous system (CNS) depressants is likely to produce additive depressant effects which can increase the risk of life-threatening respiratory depression (US FDA, 2016).

The abuse liability and dependence producing potential of isotonitazene have not been studied. However, etonitazene and metonitazene, both closely related homologues to isotonitazene, as well other members of this group, have been studied to varying degrees. Similar to other opioid analgesics, these studies suggest that members of the 2-benzylbenzimidazole group of opioids, including isotonitazene, are likely to have an abuse liability and dependence-producing potential in humans.

4.1 Pharmacodynamics

4.1.1 *In vitro* data

Data on the effect of isotonitazene on MOP (μ) receptors *in vitro* have recently been published (Table 2) (Blanckaert et al., 2020). The MOP receptor agonist activity in a live cell-based reporter assay of an isotonitazene sample purchased from an online vendor was essentially identical with that of an isotonitazene reference standard (Cayman Chemical, 2020a) indicating its high purity. Receptor activation potency (EC_{50}) and efficacy (E_{max}) data show that, similar to fentanyl, isotonitazene is a highly potent, full MOP receptor agonist *in vitro*. The opioid receptor antagonist naloxone antagonised MOP receptor activation by isotonitazene. There are no comparative data available for etonitazene or metonitazene, both closely related homologues of isotonitazene, from this study.

TABLE 2

Potency (EC_{50}) and efficacy (E_{max}) of hydromorphone, isotonitazene reference standard (from Cayman Chemical (2020a)), and an isotonitazene sample (from an online vendor) in an opioid receptor activation assay. Data for fentanyl is also shown but from unpublished results using the same assay (Blanckaert et al., 2020).

Compound	EC_{50} (nM)	E_{max} (%)
Hydromorphone	26.3 (22.0–30.7)	100 (97.3–103)
Fentanyl	18.7 (15.1–23.3)	155 (149–161)
Isotonitazene (reference standard)	11.1 (9.10–13.6)	180 (174–186)
Isotonitazene (online vendor)	12.9 (11.7–14.3)	183 (180–187)

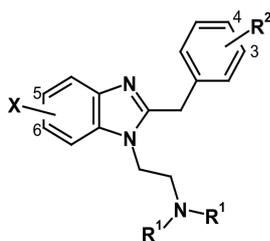
No information is available on any potential activity of isotonitazene at other pharmacological target.

4.1.2 *In vivo* data

Subcutaneously injected isotonitazene was 500 times more potent as morphine as an antinociceptive drug in the mouse tail-flick assay (radiant heat method) (Hunger *et al.*, 1960b) (code number XLII in Table 3). Of the substances reported so far in this series isotonitazene was the second most active after etonitazene. The tail-flick assay is a useful assay for discriminating between centrally acting morphine-like analgesics and non-opiate analgesics (Daniel, 2016).

TABLE 3

Antinociceptive potencies of isotonitazene, etonitazene and metonitazene, both closely related homologues to isotonitazene, as well clonitazene, relative to 5 mg/kg subcutaneously (sc) or 25 mg/kg orally administered morphine in mice; sc injection to rats relative to 2 mg/kg morphine sc, and iv injection to rabbits relative to 3 mg/kg morphine iv. Potency was determined by measuring delay in reaction time to radiant heat stimulation of the mouse tail (tail-flick), by the tail pressure method in rats and by the ear-pinch reflex method in the rabbit. Codes in Arabic numerals refer to those in the original publications (Hunger et al., 1957; Gross and Turrian, 1957). The substance with a Roman numeral code is isotonitazene and is from a subsequent publication (Hunger et al., 1960b; Ujváry, 2020).



Code number	Common name (if any)	R ¹	R ²	X	Relative potency			
					mouse sc	mouse oral	rat sc	rabbit iv
	morphine		not applicable		1	1	1	1
8	clonitazene	CH ₂ CH ₃	4-Cl	5-NO ₂	3	5	1	10
13	metonitazene	CH ₂ CH ₃	4-OCH ₃	5-NO ₂	100	15	30	200
14	etonitazene	CH ₂ CH ₃	4-OCH ₂ CH ₃	5-NO ₂	1000	1250	1000	1000
XLII	isotonitazene	CH ₂ CH ₃	4-OCH(CH ₃) ₂	5-NO ₂	500		No data	

4.2 Psychological and behavioural effects

The psychological and behavioural effects of isotonitazene have not been studied. Based on the limited information on the pharmacological properties of isotonitazene, as well information from the study of etonitazene and metonitazene, and other substances from this group (Ujváry, 2020), it is likely that the effects of isotonitazene share some similarities with those commonly reported for other opioid analgesics, including: dizziness, drowsiness, mental confusion, incoordination, relaxation, and euphoria; at higher doses, sedation and profound intoxication would be expected.

4.3 Safety pharmacology

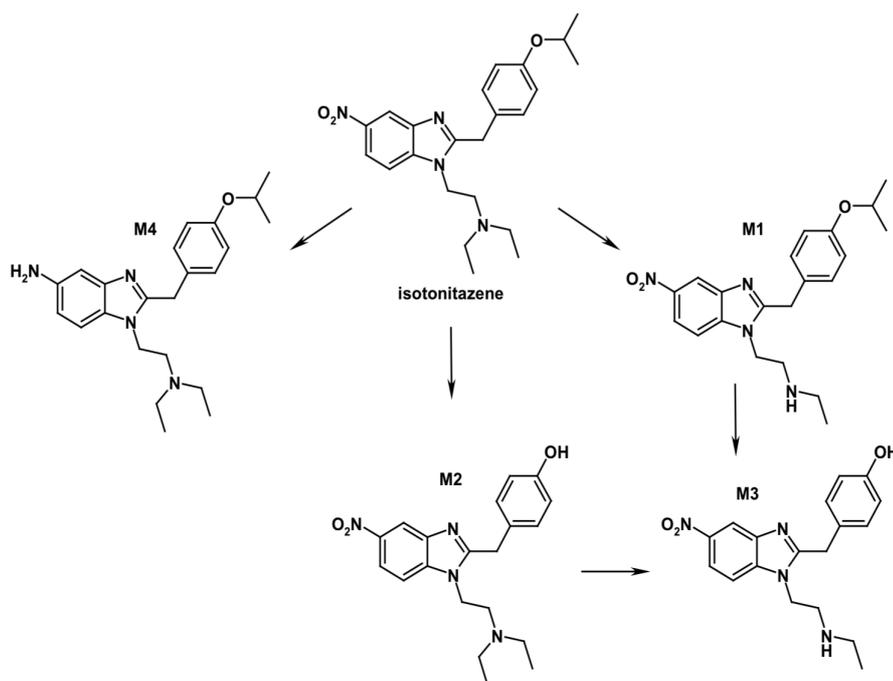
The safety pharmacology of isotonitazene has not been studied. Based on the limited information on the pharmacological properties of isotonitazene, as well information from the study of etonitazene and metonitazene, and other substances from this group, and, similar to other opioid analgesics, the most serious acute risk from the use of isotonitazene in humans is likely to be from dose-dependent

respiratory depression, which can lead to apnoea, respiratory arrest, and death (Pattinson, 2008; Romberg et al., 2003; White and Irvine, 1999).

4.4 Pharmacokinetics

The pharmacokinetics of isotonitazene has not been studied. However, a recent publication that describes a series of 18 death cases with confirmed exposure to isotonitazene in the United States identified several common metabolites from urine and blood (Krotulski *et al.*, 2020). The proposed metabolic scheme is shown in Figure 3. The principal urinary metabolites were the *N*-dealkylated product, the tertiary amine affording the corresponding secondary amine derivative of isotonitazene (*N*-desethyl-isotonitazene or 'nor-isotonitazene'; M1) and the *N*-dealkyl-*O*-dealkyl species (*N*-desethyl-*O*-desisopropyl-isotonitazene; M3). The 5-amino metabolite (M4), arising from the reduction of the 5-nitro group, was detected in the majority of blood samples. Urinary metabolites M1 and M3 appear to be most suitable for monitoring the ingestion of isotonitazene. The *O*-dealkylated metabolite M3 could also be a useful marker of etonitazene and metonitazene ingestion as well.

FIGURE 3
Proposed metabolism of isotonitazene (Krotulski *et al.*, 2020)



It is noted that the phenolic M2 was reported to be equipotent with morphine (Hunger *et al.*, 1960b). Although the reduced amine M4 has not been reported, the 4-ethoxy analogue, a potential metabolite of etonitazene, is twice as potent as morphine (Hunger *et al.*, 1960b). Nevertheless, the contribution of metabolites M2 and M4 to the overall effects *in vivo* of isotonitazene or close etonitazene analogues should be negligible. There are no pharmacology data on metabolites M1 and M3.

The *N*-desethyl metabolite (M1) was also detected in urine and blood samples from a death case with confirmed exposure to isotonitazene reported by the United Kingdom (Section 5.4; Section 6.2.2) (EMCDDA, 2020a).

4.5 Toxicology

The toxicology of isotonitazene has not been studied. However, the acute toxicity of etonitazene and metonitazene, both closely related homologues to isotonitazene, as well other members of this group, has been studied in mice and rabbits (Gross and Turrian, 1957; Ujvary, 2020). The LD₅₀ values in mice, and the 'medium lethal dose' in rabbits with or without urethane narcosis⁽¹⁶⁾ as well as respiratory depression in rabbits pretreated with urethane for etonitazene, metonitazene, as well as clonitazene are shown in Table 4. Mechanical ventilation or the respiratory stimulants nikethamide and bemegride counteracted respiratory depression. Of note is that pretreatment of rabbits with the narcotic anaesthetic urethane increased the toxicity of the compounds by several orders of magnitude.

Despite the lack of experimental data, observations that antinociceptive potency in general correlates with acute toxicity within this group of substances suggest that toxicity of isotonitazene may be similar to that estimated for metonitazene or etonitazene.

TABLE 4

Toxic effects of selected 2-benzylbenzimidazole opioids in different laboratory animals upon oral (mouse) or intravenous (mouse and rabbit) administration. For rabbits, acute toxicity was assessed either with or without urethane-pretreatment (1.4 g/kg subcutaneous); the effect on respiration was studied with urethane-pretreatment (1.4 g/kg subcutaneous) (Gross and Turrian, 1957).

Compound	Mouse LD ₅₀ (mg/kg)		Rabbit toxicity (all doses in mg/kg)		
	iv	oral	Median lethal dose without urethane	Median lethal dose urethane pretreatment	Dose decreasing respiration frequency by 50%
Morphine	200	1000	250	1.0	0.5
Clonitazene	50	100	25	0.25	0.17
Metonitazene	50	100	50	0.025	0.010
Etonitazene	1	25	0.5	0.0025	0.0005
Isotonitazene			No data		

While no opioid antagonist was used in this particular study, subsequent experiments with isolated tissues (Gyang et al., 1964; Hughes et al., 1975), receptor studies *in vitro* (Pert and Snyder, 1973) and experiments *in vivo* (Barnett et al., 1975; Aceto et al., 1994; Achat-Mendes et al., 2009) indicated that opioid antagonists counteract etonitazene-induced effects. Importantly, during a human clinical trial, respiratory depression and coma caused by 1 mg subcutaneously administered metonitazene could be antagonised by prompt intravenous injection of 5 mg of the opioid antagonist nalorphine

⁽¹⁶⁾ Urethane (ethyl carbamate) is a narcotic anaesthetic and was used at a 1.4 g/kg subcutaneous dose in this case.

(Bromig, 1958). More specifically, activation of a MOP receptor preparation *in vitro* by isotonitazene was antagonised by the opioid antagonist naloxone (Blanckaert et al., 2020).

Based on the available information for isotonitazene and related substances, and similar to other opioid analgesics, the most serious acute health risk from isotonitazene is likely to be respiratory depression, which in overdose could lead to apnoea, respiratory arrest, and death (Pattinson, 2008; Romberg et al., 2003; White and Irvine, 1999).

Similarly to other opioid analgesics, the use of isotonitazene with other central nervous system (CNS) depressants, including other opioids, sedatives/hypnotics, alcohol, pregabalin, gabapentin, tranquillisers, and sedating anti-histamines, is likely to produce additive depressant effects which can increase the risk of life-threatening respiratory depression, arrest, and death (US FDA, 2016).

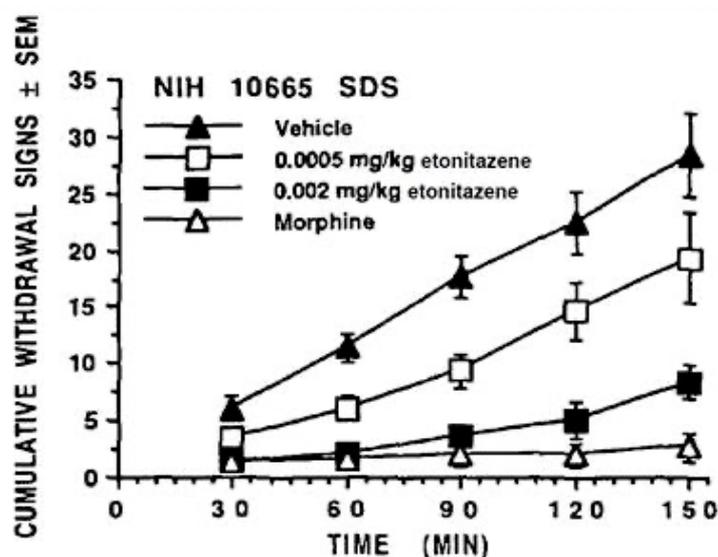
4.6 Abuse liability and dependence producing potential

The abuse liability and dependence producing potential of isotonitazene have not been studied. However, the abuse liability and dependence producing potential of etonitazene and metonitazene, both closely related homologues to isotonitazene, as well other members of this group, have been studied to varying degrees (Ujváry, 2020).

Information on etonitazene

In morphine-addicted rhesus monkeys which had received 3 mg/kg morphine for at least 3 months, subcutaneously injected etonitazene was 1500-fold while clonitazene was only two-fold more potent than morphine sulfate in suppressing abstinence signs; the onset was rapid but the duration of action of both of these benzimidazoles was less than that of morphine. At the highest dose (0.002 mg/kg), jaw and body sag, scratching and rubbing face were noted (Deneau et al., 1959; Aceto et al., 1992). The results of a single-dose substitution (SDS) test with morphine (3.0 mg/kg) and etonitazene (0.0005 mg/kg and 0.002 mg/kg) subcutaneous doses in rhesus monkeys are shown in Figure 4.

FIGURE 4
Single-dose substitution of morphine by two doses of etonitazene in rhesus monkeys showing withdrawal signs after abrupt cessation of chronic morphine treatment (Aceto et al., 1992).



The reinforcing properties of etonitazene have been demonstrated in several animal models. For example, in rhesus monkeys, orally administered etonitazene was established as a positive reinforcer (Carroll and Meisch, 1978).

In self-administration experiments with rhesus monkeys (Achat-Mendes et al., 2009), etonitazene and heroin appeared to have different reinforcing properties: heroin was consistently self-injected (0.001–0.01 mg/kg dose range) while etonitazene failed to maintain significant levels of self-administration (0.001–1.0 µg/kg dose range ⁽¹⁷⁾). It was however noted that self-administration of an etonitazene + cocaine combination maintained a significantly greater number of injections than did cocaine alone. Furthermore, the MOP receptor antagonist naltrexone blocked self-administration of both etonitazene+cocaine and heroin+cocaine combinations, but not of cocaine self-administration suggesting that MOP receptor mechanisms are important in mediating the ‘speedball’ effect.

In a human pain-treatment clinical study with clonitazene administered either by injection (repeated 15–30 mg subcutaneous or intramuscular doses) or orally (repeated 50 mg doses), tolerance did not appear to have developed after a 35-day continuous treatment and no craving was observed in the patients (Bromig, 1958).

In stabilised morphine addicts, one mg etonitazene orally was equivalent to 59.3 mg morphine subcutaneously (about 60-fold difference) as suppressant of abstinence phenomena (Eddy, 1959).

The addictive potential in man of orally administered clonitazene and etonitazene, as compared to morphine, was studied in non-tolerant former morphine addicts at the Addiction Research Center, US Public Health Service Hospital of the US National Institute of Health (Fraser et al., 1960; Wikler, 1960). In ‘single dose’ oral administrations, clonitazene (NIH-7586; 100 mg) appeared to be about one-third to one-fifth as potent as morphine sulfate (20 and 30 mg) and roughly equivalent to codeine (60 or 90 mg), while etonitazene (NIH-7607; 0.25 mg) was more than 80 to 120 times as effective as morphine as a euphoriant. Both clonitazene and etonitazene caused miosis. Furthermore, both drugs suppressed abstinence symptoms: 2.62 mg of clonitazene orally was equivalent to 1 mg of morphine given subcutaneously while 1 mg of etonitazene orally, was equivalent to 59.3 mg morphine subcutaneously. In double-blind direct addiction tests involving 8 patients, orally administered etonitazene was not always classified as subcutaneously injected morphine- or heroin-like; in some cases effects resembling barbiturates were mentioned. Though patients reported preference to morphine or heroin over etonitazene, they were impressed by the hypnotic actions of etonitazene. Abrupt discontinuation following repeated administration of etonitazene, morphine or heroin, ‘moderately severe abstinence syndrome ensued during the next ten days’. The study concluded that both clonitazene and etonitazene have addictive potential comparable to that of morphine.

It is of note that that no euphoria was observed in patients receiving clonitazene 30 mg parenteral or 50 mg oral doses during a pain treatment trial (Bromig, 1958).

In 1960, in their assessment etonitazene and clonitazene the World Health Organization (WHO) Expert Committee on Addiction-Producing Drugs stated their ability to produce morphine-like effects, to suppress abstinence phenomena of a known morphine addiction, as well as to sustain morphine addiction, and recommended both drugs to be placed under international control (ECAPD, 1961).

⁽¹⁷⁾ Doses above 1.0 µg/kg cause significant respiratory depression in monkeys (Butelman et al., 1993).

Tolerance, or dose escalation in illicit opioid users in particular, apparently develops for all opioids and this observation extends to etonitazene (Walker and Young, 2001; Morgan and Christie, 2011).

Information on metonitazene

In morphine-addicted monkeys, subcutaneously injected metonitazene was a hundred-times more potent than morphine sulfate (0.03 mg/kg versus 3 mg/kg) in suppressing abstinence signs; the duration of action is about one-half that of morphine (Deneau et al., 1959; Ujvary, 2020).

5. Extent and patterns of use, availability, and potential for diffusion

5.1 Summary

There is limited information on the extent and patterns of use, availability, and potential for diffusion of isotonitazene in Europe.

Isotonitazene has been available on the drug market in Europe since at least April 2019, when it was seized for the first time by police in Estonia. The most recent identification of isotonitazene reported to the EMCDDA is a seizure made by police in Latvia in January 2020. As of 1 May 2020, it has been identified in six Member States: Belgium, Estonia, Germany, Latvia, Sweden, and the United Kingdom. These detections relate to police seizures reported by Estonia, Germany, and Latvia; a customs seizure reported by Sweden; a collected sample reported by Belgium; and biological samples from a death case reported by the United Kingdom. While the seized quantities are relatively small, they should be seen within the context of the possible high potency of isotonitazene (Section 4.1).

It appears that at least some of the isotonitazene on the market in Europe has been produced by chemical companies based in China, although the size and scale of this production is unknown. Isotonitazene is sold online as a powder in wholesale and small amounts; it is also sold as ready-to-use nasal sprays. Based on limited information from police seizures reported from two Member States (Estonia and Latvia) it is presumed, but not confirmed, that isotonitazene has been sold on the illicit opioid market at street-level and being injected intravenously by people who use opioids.

Isotonitazene may be deliberately sought after by some users, such as those who self-experiment with a range of psychoactive substances (so-called 'psychonauts') (Section 2.6); others, such as those that purchase it at street-level, may be unaware that they are using the substance which presents an inherent risk to the individuals. Although the drug situation is different from Europe, information from a case series of 18 deaths with confirmed exposure to isotonitazene in the United States found that at least some of the individuals were high risk drug users and included people who had a history of injecting opioids such as heroin (Krotulski et al., 2020; Power, 2020). In addition, isotonitazene was identified along with one or more other psychoactive substances (controlled drugs and new psychoactive substances) in all the deaths, which suggests that polydrug use was common in these individuals (Krotulski et al., 2020). In particular, the use of other CNS depressants such as other opioids and benzodiazepine sedative/hypnotics was very common in these individuals (Section 6.2.2).

As isotonitazene has only been on the drug market for a short period of time, it may not be part of drug screening in many forensic and toxicology laboratories in Europe. Therefore, the presence of isotonitazene on the European drug market may be undetected in some areas, including in law

enforcement seizures as well as in biological samples related to serious adverse events. Furthermore, analytical sensitivity related to the analysis of biological samples from serious adverse events may also be a potential issue as the concentration of isotonitazene or its metabolites can be sub-nanogram to picogram (similar to the highly potent opioid carfentanil) which highlights a need for increased analytical sensitivity when testing for the substance. In addition, due to differences in reporting practices across Europe, identifications of isotonitazene may be unreported to the Reitox national focal points, and, as a result, to the EMCDDA. It is also important to note that, in some settings, there may be delays in detecting and reporting detections of isotonitazene due to reduced capacity of early warning systems, including forensic science and toxicology laboratories, as a result of the impact of the ongoing coronavirus disease (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (ECDC, 2020; WHO, 2020).

More generally, the impact of the ongoing COVID-19 pandemic (ECDD, 2020; WHO, 2020) on the manufacture, trafficking, distribution, and use of isotonitazene is currently unknown. Based on previous experiences with disruptions to the illicit opioid markets (Ciccarone, 2019; EMCDDA, 2011; EMCDDA, 2012; Mars et al., 2019), it is conceivable, that, should there be a reduced availability of established illicit opioids in Europe, such as heroin as well as fentanyl and its derivatives, then criminal groups, as well as people who use opioids, especially high risk opioid users, may substitute these substances for a range of other substances, including 2-benzylbenzimidazole opioids such as isotonitazene. These changes may be geographically localised or broader, they may also be single 'one off' events, or short-lived, or longer lasting changes. Similar to the recent experience with highly potent fentanyl derivatives, such as acryloylfentanyl and carfentanil, such changes to the drug market may increase the risk of life-threatening poisoning, as, currently, there is little to no experience with the use of isotonitazene (such as how to dose the substance and the effects it can cause); in some cases such substitution could manifest as outbreaks of poisoning (EMCDDA, 2018; Evans-Brown and Sedefov, 2018; Ujváry et al., 2017). These risks will be especially high in the case of people who obtain isotonitazene from street-level illicit opioids markets who are unlikely to be aware that they are using the substance.

5.2 Information from seizures

Law enforcement seizures of isotonitazene have been reported to the EMCDDA by four Member States: Estonia, Latvia, Germany, and Sweden. In total, 24 seizures were reported (1 made by customs; 23 made by police). In 22 cases isotonitazene was seized in powder form (total of 109.6 g of powder) and in two cases in liquid form (4.5 g). Where known, the seizures took place between April 2019 and January 2020.

5.2.1 Customs seizures

Sweden reported a seizure of isotonitazene that was made by customs. The seizure took place in September 2019. A total of 48.8 g of powder was seized; the colour was described as yellow. Information on the amount of isotonitazene present in the seized powder (chemical purity) was not reported. The seized package originated from China and the destination was Sweden.

5.2.2 Police seizures

A total of 23 seizures of isotonitazene made by police were reported three Member States: Estonia (n=17), Latvia (n=4), and Germany (n=2) ⁽¹⁸⁾. Where known, the seizures took place between April 2019 and January 2020.

In 21 cases, isotonitazene was seized in powder form totalling 60.8 g (range 0.013 to 19.8 g); the colour of the powder was described as brown in some of the cases reported by Estonia. Information on the amount of isotonitazene present in the seized powders (chemical purity) was not reported. Estonia reported that the only other substances detected in the powders were 'common sugars' (not further specified); Latvia reported that one seizure also contained fentanyl. Estonia reported that some of the seizures related to small-scale distribution/supply. In one case reported by Latvia, powders of isotonitazene were found in 19 individual foil packages also suggesting small-scale distribution/supply.

In two cases reported by Germany, the seized products were liquids (product name: 'ISOTONITAZEN EXTRA STRONG') and also contained trace amounts of the synthetic cannabinoid 5F-MDMB-P7AICA. Information on the amount of isotonitazene present in the seized liquids (strength) was not reported.

5.3 Information from collected samples

Belgium reported a collected sample of isotonitazene to the EMCDDA. It was a test purchase made by the national focal point via a private Telegram-group linked to a private website. The sample was incorrectly advertised as 'etonitazene'. The cost was \$400 per 1 g. It was received as a white powder in a plastic zip-lock bag which was then packaged inside a foil bag, with the label 'iso'. Based on the analyses conducted it was reported that the sample was of 'high purity' (Blanckaert et al., 2020).

5.4 Information from biological samples

The United Kingdom reported post-mortem biological samples (blood and urine) to the EMCDDA from a death in which isotonitazene was identified. Butyrylfentanyl, despropionyl fentanyl (4-ANPP), and despropionyl fluorofentanyl were also identified in the biological samples. All the substances were estimated to be at sub ng/ml levels. The death occurred in 2019; no further details are currently available (Section 6.2.2).

6. Health risks

6.1 Summary

Based on the available information for isotonitazene the most serious acute health risk from isotonitazene is likely to be respiratory depression, which in overdose could lead to apnoea, respiratory arrest, and death (Pattinson, 2008; Romberg et al., 2003; White and Irvine, 1999). Although there is no reported clinical experience with poisonings cases by isotonitazene, based on

⁽¹⁸⁾ Seizures of isotonitazene made by police have also been reported in Canada. Physical forms seized include powders and falsified (fake) opioid analgesic medicines (Section 2.6).

the pharmacological properties of the substance, naloxone is expected to work as an antidote (Boyer, 2012; Kim and Nelson, 2015). Similarly to other opioids analgesics, the use of isotonitazene with other central nervous system (CNS) depressants, is likely to produce additive depressant effects which can increase the risk of life-threatening respiratory depression and arrest (US FDA, 2016).

A total of 2 deaths involving isotonitazene have been reported to the EMCDDA. The role of isotonitazene in these deaths is unknown.

There is no information on the chronic health effects of isotonitazene, including abuse liability and dependence production potential. Similar to established opioids, the chronic health risks might share some similarities to those seen with established illicit opioids, such as heroin and fentanyl. This may include dependence.

6.2 Acute health effects

Based on the available information for isotonitazene, as well as for etonitazene and metonitazene, both closely related homologues to isotonitazene, as well other members of this group, and, similar to other MOP receptor agonists, the most serious acute health risk from isotonitazene is likely to be respiratory depression, which in overdose could lead to apnoea, respiratory arrest, and death (Pattinson, 2008; White and Irvine, 1999). This risk may be greater due to the fact that isotonitazene is the first of the 2-benzylbenzimidazole opioids to be identified on the drug market in recent years, and users have no experience with this family of opioids, including a lack of information on what doses to use and what effects the substance can have.

Although there is no information on the clinical features of poisoning caused by isotonitazene, they are likely to include the opioid overdose triad of miosis, reduced level of consciousness or unconsciousness, and respiratory depression.

Although there is no reported clinical experience with poisonings caused by isotonitazene, based on the pharmacological properties of the substance, naloxone is expected to work as an antidote in reversing respiratory depression (Boyer, 2012; Kim and Nelson, 2015).

Similarly to other opioids analgesics, the use of isotonitazene with other central nervous system (CNS) depressants, including other opioids, sedatives/hypnotics, alcohol, pregabalin, gabapentin, tranquillisers, and sedating anti-histamines, is likely to produce additive depressant effects which can increase the risk of life-threatening respiratory depression and arrest (US FDA, 2016). Information from Europe on polydrug use with isotonitazene is limited to a single death case reported by the United Kingdom where other opioids, specifically fentanyl derivatives, were identified. Although the drug situation is different from Europe, information from a case series of 18 deaths in the United States found that polydrug use among these individuals was common, including a majority who used one or more CNS depressant, especially other opioids and benzodiazepine sedative/hypnotics.

6.2.1 Acute poisonings

No cases of acute poisoning involving isotonitazene have been reported to the EMCDDA or published in the scientific literature.

6.2.2 Deaths

Deaths reported in Europe

A total of two deaths involving isotonitazene have been reported to the EMCDDA by Germany and the United Kingdom. No further information is currently available for the death reported by Germany. The United Kingdom reported a death with confirmed exposure to isotonitazene that occurred in 2019. Alongside isotonitazene, butyrylfentanyl, despropionyl fentanyl (4-ANPP), and despropionyl fluorofentanyl were also identified in postmortem biological samples; all the substances were estimated to be below ng/ml concentrations. No further details on the case are currently available. The role of isotonitazene in these deaths is unknown.

Deaths reported elsewhere

Canada

Isotonitazene has been identified in 3 deaths in Canada during 2019. The deaths occurred in Alberta in March, September, and October 2019. No further details are available (Toxicovigilance Canada, 2019). The role of isotonitazene in these deaths is unknown.

United States

Krotulski et al. (2020) have recently published a report of 18 deaths with confirmed exposure to isotonitazene in the United States; all cases were medico-legal death investigations. The role of isotonitazene in these deaths is unknown.

The deaths occurred between August 2019 and January 2020 and were from the Midwestern United States: Illinois (n=9), Indiana (7), Minnesota (1), and Wisconsin (1). The majority of individuals were male (n=12; 67%). The mean age was 41±12 years (median 41 years) and ranged from 24 to 66 years.

The mean concentration of isotonitazene in blood samples (N=18) was 2.2±2.1 ng/mL (median 1.75 ng/mL, range 0.4-9.5 ng/mL) (central or peripheral blood). The lowest concentration of isotonitazene in blood was 0.4 ng/mL and was encountered in two cases in which no other opioids were present (case 8 and case 10). The mean concentration of isotonitazene in urine samples (N=6) was 2.4±1.4 ng/mL (median 2.7 ng/mL, range 0.6-4.0 ng/mL). The concentration of isotonitazene in a single sample of vitreous fluid was 0.1 ng/mL.

Isotonitazene was identified in all the biological samples that were analysed in this study (blood, urine, vitreous fluid), which may suggest the usefulness of this analyte for drug testing purposes. It is important to note that, similar to the highly potent opioid carfentanil, quantitative results for isotonitazene were low to sub-nanogram per millilitre concentrations, highlighting the need for increased analytical sensitivity when testing for the substance. Details on the identification and characterisation of metabolites in blood and urine are also provided in the report (Krotulski et al., 2020) (Section 5.4).

Based on information from the death investigations (n=8) and forensic toxicology results (n=18), at least some of the individuals were high risk drug users and included people who had a history of injecting established illicit opioids, such as heroin and fentanyl. Isotonitazene was identified along with one or more other psychoactive substances (controlled drugs and new psychoactive substances)

in all the deaths, which suggests that polydrug use was common in these individuals. Briefly, among other findings in blood:

- In addition to isotonitazene, one or more other types of opioids were identified in 10 (56%) of the cases; these included fentanyl (n=6), morphine (n=3) (including 6-monoacetylmorphine in one case), tramadol (n=4), piperidylthiambutene (n=2), and U-47,700 (n=1). Isotonitazene was the only opioid identified in the remaining 8 cases.
- Benzodiazepines were identified in 16 (89%) of the cases; most of the identifications related to flualprazolam (n=11) or etizolam (n=8) ⁽¹⁹⁾.
- Stimulants were identified in 6 (33%) of the cases: cocaine was identified in 4 (22%) cases; amphetamine and methamphetamine were identified in 2 (11%) cases (in one of these cases MDMA and MDA were also identified).
- Naloxone (an antidote used in opioid overdose) was identified in 6 (33%) of the cases. This includes 2 cases where isotonitazene was the only opioid identified.

6.2.3 Driving and operating machinery under influence

The effect of isotonitazene on the ability to drive and operate machinery has not been studied. However, it is well established that opioid analgesics impair the mental and physical ability to drive and operate machinery. This effect is likely to extend to isotonitazene.

6.3 Chronic health effects

There is no information on the chronic health effects of isotonitazene. Similar to established opioids, the chronic health risks might share some similarities to those seen with established illicit opioids, such as heroin and fentanyl. This may include dependence (Section 4.6).

Based on the limited information reported to the EMCDDA from police seizures, it is presumed, but not confirmed, that isotonitazene is being injected intravenously by high risk opioid users in some parts of Europe. Although the drug situation is different from Europe, information from a case series of 18 deaths with confirmed exposure to isotonitazene in the United States noted that at least some of the individuals were high risk drug users and included people who had a history of injecting opioids such as heroin (Krotulski et al., 2020). As such, and similar to the use of established illicit opioids, there is a risk of transmission of blood-borne infections, such as HIV, hepatitis B, and hepatitis C, if injecting equipment is shared (Degenhardt et al., 2016; EMCDDA, 2020b).

7. Social risks

There have been no studies on the social risks of isotonitazene. Given that isotonitazene acts as an opioid analgesic, any such risks may have some similarities with those associated with established illicit opioids such as heroin as well as fentanyl and its derivatives.

⁽¹⁹⁾ Flualprazolam and etizolam are monitored by the EMCDDA as new psychoactive substances. The substances were recently controlled under the United Nations Convention on Psychotropic Substances, 1971, over concerns of the public health threats that they pose (UNODC, 2020).

7.1 Individual social risks

There is no information on the individual social risks that may be associated with the use of isotonitazene. Given that isotonitazene acts as an opioid analgesic, any such risks may have some similarities with those associated with established illicit opioids. These risks may negatively impact on education or career, family or other personal and social relationships and may result in marginalisation.

7.2 Possible effects on direct social environment

There is no information on the possible effects of isotonitazene on the direct social environment. Given that isotonitazene acts as an opioid analgesic, any such effects may have some similarities with those associated with the use of established illicit opioids.

7.3 Possible effects on society as a whole

There is no information on the possible effects of isotonitazene on society as a whole. Given that isotonitazene acts as an opioid analgesic, any such effects may have some similarities with those associated with the use of established illicit opioids.

7.4 Economic costs

There are no information on the health and social costs related to isotonitazene. Given that isotonitazene acts as an opioid analgesic, any such costs may have some similarities with those associated with the use of established illicit opioids.

7.5 Possible effects related to the cultural context, for example marginalisation

There is no information on the possible effects of isotonitazene related to the cultural context. Given that isotonitazene acts as an opioid analgesic, any such effects may have some similarities with those associated with the use of established illicit opioids.

7.6 Possible appeal to specific population groups within the general population

Based on limited information, alongside what appears to be small-scale interest in isotonitazene by people who self-experiment with psychoactive substances (so-called 'psychonauts'), it is presumed, but not confirmed, that isotonitazene is being injected intravenously by high risk opioid users in some parts of Europe. Although the drug situation is different from Europe, information from a case series of 18 deaths with confirmed exposure to isotonitazene in the United States noted that at least some of the individuals were high risk drug users and included people who had a history of injecting opioids such as heroin (Krotulski et al., 2020). Based on this information and that isotonitazene acts as an opioid analgesic, it is reasonable to assume that isotonitazene may be sought by those looking for 'legal' substitutes for established illicit opioids, such as heroin, as well as fentanyl and its derivatives, and/or prescription opioids.

7.7 Involvement of criminal groups in the manufacture, distribution and distribution methods, and trafficking

There is no information on whether or not criminal groups are involved in the manufacture, trafficking, and distribution of isotonitazene within Europe (EMCDDA, 2020a). The impact of the ongoing COVID-19 pandemic (ECDD, 2020; EMCDDA, 2020b; WHO, 2020) on the manufacture, trafficking, distribution, and use of isotonitazene is also currently unknown. Based on previous experiences with disruptions to the illicit opioid markets (Ciccarone, 2019; EMCDDA, 2011; EMCDDA, 2012; Mars et al., 2019), it is conceivable, that, should the availability of established illicit opioids, such as heroin and/or fentanyl and its derivatives, be reduced in Europe, then criminal groups, as well as people who use opioids, especially high risk opioid users, may substitute these substances for a range of other substances, including 2-benzylbenzimidazole opioids such as isotonitazene.

8. Other relevant information

8.1 Information on restrictive measures

8.1.1 International restrictive measures

At international level, Isotonitazene is not controlled under the United Nations Single Convention on Narcotic Drugs, 1961, as amended by the 1972 Protocol, or the Convention on Psychotropic Substances of 1971 ('United Nations system') (UNODC, 2019a; UNODC, 2019b). Isotonitazene has not been subject to assessment nor is it currently under assessment by the United Nations system (EMCDDA, 2020a).

8.1.2 National restrictive measures

Twenty two Member States (Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Luxembourg, Malta, the Netherlands, Portugal, Romania, Slovakia, Slovenia, and Spain) reported that isotonitazene is not subject to restrictive measures at national level. Turkey also reported that isotonitazene is not subject to restrictive measures at national level (EMCDDA, 2020a).

Isotonitazene is subject to restrictive measures in six Member States: in Estonia, Latvia, Poland, and Sweden the substance is controlled under drug control legislation; in Lithuania it is controlled under medicines legislation; while in the United Kingdom it is controlled by new psychoactive substance legislation. In addition, isotonitazene is controlled under medicines legislation in Norway (EMCDDA, 2020a).

It is unknown if isotonitazene is controlled in China, where at least some of the substance on the European market has been sourced from.

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ANNEX 2

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The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) is the central source and confirmed authority on drug-related issues in Europe. For over 25 years, it has been collecting, analysing and disseminating scientifically sound information on drugs and drug addiction and their consequences, providing its audiences with an evidence-based picture of the drug phenomenon at European level.

The EMCDDA's publications are a prime source of information for a wide range of audiences including: policymakers and their advisors; professionals and researchers working in the drugs field; and, more broadly, the media and general public. Based in Lisbon, the EMCDDA is one of the decentralised agencies of the European Union.

Related publications and websites**EMCDDA**

| EMCDDA Initial Report on *N,N*-diethyl-2-[[4-(1-methylethoxy)phenyl]methyl]-5-nitro-1*H*-benzimidazole-1-ethanamine (isotonitazene), 2020
https://www.emcdda.europa.eu/publications/initial-reports/isotonitazene_en

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