European Monitoring Centre for Drugs and Drug Addiction

ADVANCED RELEASE

EMCDDA technical report on the new psychoactive substance *N*,*N*-diethyl-2-[[4-(1-methylethoxy)phenyl]methyl]-5-nitro-1*H*-benzimidazole-1-ethanamine (isotonitazene)

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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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- the European Medicines Agency (EMA), the European Chemicals Agency (ECHA), the European Centre for Disease Prevention and Control (ECDC), the European Food Safety Authority (EFSA), and the European Commission;
- Peter Blanckaert, Coordinator Belgian Early Warning System Drugs (BEWSD), Sciensano, Belgium.

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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 ([1]).

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

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

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 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 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 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 ([2]) and metonitazene ([3]), as well as clonitazene ([4]). 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).

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

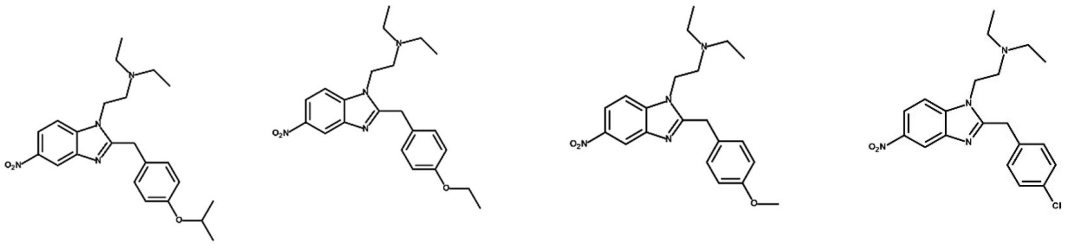
Although isotonitazene was one of 2-benzylbenzimidazole compounds reported in patents and in the original study on this group of substances (Hoffmann et al., 1959; Hoffmann et al. 1960; Hunger et al., 1960b), no additional reports related to isotonitazene could be found until its identification on the 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 ([5]).

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 halogen atom with the ethereal isopropoxy group. The chemical structure and molecular properties of these compounds are provided in Figure 1 ([6]).

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



([5]) In accordance with Council Framework Decision 2004/757/JHA (as amended) and Regulation (EC) No 1920/2006 (as amended).
(6) Another closely related substance is the n-propoxy isomer of isotonitazene, known as protonitazene (*N*,*N*-diethyl-2-[(4-propoxyphenyl)methyl]-5-nitro-1*H*-benzimidazole-1-ethanamine). Protonitazene 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 protonitazene. Information of the pharmacology of pronitazene can be found in Hunger et al., (1960b) (compound XLIII in Table I); briefly protonitazene 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 protonitazene 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.

Molecular formula: $C_{23}H_{30}N_4O_3$	Molecular formula: $C_{22}H_{28}N_4O_3$	Molecular formula: $C_{21}H_{26}N_4O_3$	Molecular formula: $C_{20}H_{23}ClN_4O_2$
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 ([7]) or miLogP were calculated by Molinspiration property engine v2018.10 ([8]) and StarDrop® Version 6.6 software, ([9]) respectively. The topological polar surface area (TPSA) ([10]) was calculated by StarDrop® Version 6.6 software.

Common name:

Isotonitazene

Systematic (IUPAC) name:

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

Other chemical names:

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

([7]) 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).
([8]) https://www.molinspiration.com/cgi-bin/properties
([9]) Optibrium Ltd, Cambridge, UK.
([10]) 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.

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

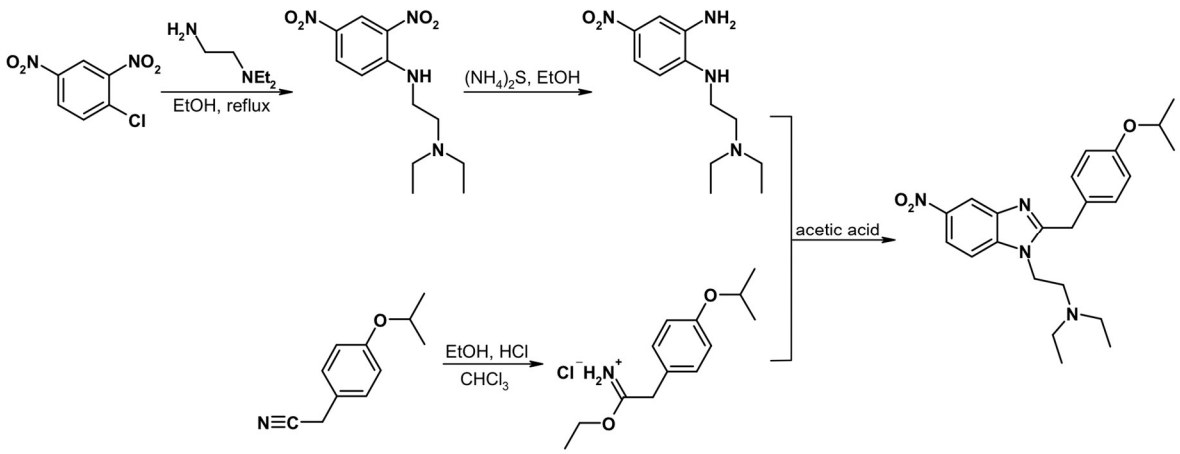
There is no information on the chemical precursors or manufacturing methods used to synthesise the isotonitazene which has been identified on the drug market in Europe.

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 [11] 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.

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

([11]) IUPAC name: *N*1-[2-(diethylaminoethyl]-4-nitrobenzene-1,2-diamine

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), ^{1}H 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 *et al.*, 2020
Ultraviolet spectroscopy	Blanckaert *et al.*, 2020 Cayman Chemical, 2020a
Infrared spectroscopy	ADEBAR, 2020 Blanckaert *et al.*, 2020
Raman spectroscopy	ADEBAR, 2020
^{1}H NMR spectroscopy	ADEBAR, 2020 Blanckaert *et al.*, 2020
^{13}C NMR spectroscopy	ADEBAR, 2020 Blanckaert *et al.*, 2020
Mass spectrometry	NPSDiscovery, 2019 ADEBAR, 2020 Blanckaert *et al.*, 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 ([12]), 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 ([13]) used for isotonitazene is limited. Isotonitazene ([14]) 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 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,

([12]) IUPAC name for protonitazene: *N*,*N*-diethyl-2-[(4-propoxyphenyl)methyl]-5-nitro-1*H*-benzimidazole-1-ethanamine
([13]) 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.
([14]) 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.

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} (%)

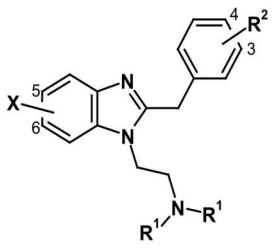
Hydromorphone	26.3 (22.0–30.7)	100 (97.3–103)
Fentanyl	18.7 (15.1–23.3)	155 (149–161)
Isotonitazene (reference standard)	11.1 (9.10–13.6)	180 (174–186)
Isotonitazene (online vendor)	12.9 (11.7–14.3)	183 (180–187)

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		rat	rabbit
					sc	oral	sc	iv
	morphine	not applicable			1	1	1	1
8	clonitazene	CH_2CH_3	4-Cl	5-NO_2	3	5	1	10
13	metonitazene	CH_2CH_3	4-OCH_3	5-NO_2	100	15	30	200
14	etonitazene	CH_2CH_3	4-OCH_2CH_3	5-NO_2	1000	1250	1000	1000
XLII	isotonitazene	CH_2CH_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.

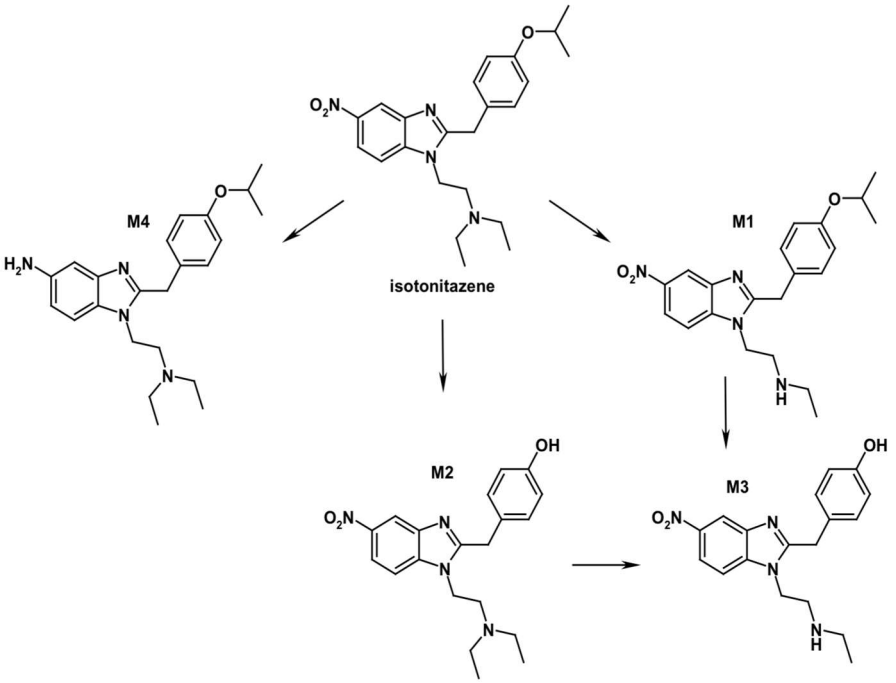
4.3 Safety pharmacology

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 LD_{50} values in mice, and the 'medium lethal dose' in rabbits with or without urethane narcosis ([15]) 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.

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 (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).

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)		
			Median lethal dose		Dose decreasing respiration frequency by 50%
	iv	oral	without urethane	urethane pretreatment	

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

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				

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).

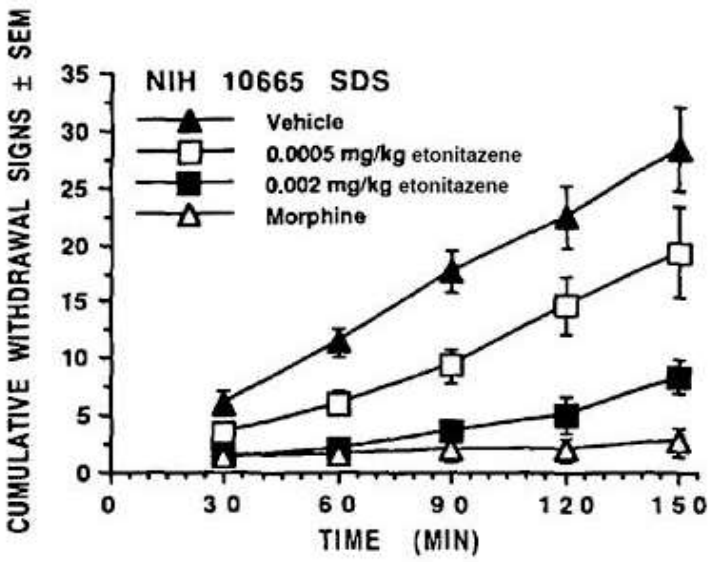
4.6 Abuse liability and dependence producing potential

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 ([16])). 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).

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

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).

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) ([17]). 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

([17]) 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).

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 jsotonitazene, based on 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) ([18]).

- 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.

([18]) 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 of 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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