Critical Review Report:
ISOTONITAZENE

Expert Committee on Drug Dependence

Forty-third Meeting

Geneva, 12–20 October 2020

This report contains the views of an international group of experts, and does not necessarily represent the decisions or the stated policy of the World Health Organization.

Contents

Executive summary

Isotonitazene is a 5-nitro-2-benzylbenzimidazole belonging to the 2-benzylbenzimidazole group of compounds synthesized more than 60 years ago as potential analgesics. However, these compounds were never clinically approved for marketing. The closely related homologues, etonitazene and clonitazene, have been scheduled internationally. The synthetic opioid isotonitazene has recently appeared on the illicit market. It has been identified in postmortem forensic toxicology reports and in national and international drug seizures beginning in April 2019. Isotonitazene has appeared in Belgium, Canada, Estonia, Germany, Latvia, Sweden, the United Kingdom and the United States of America (USA).

In vitro radioligand binding and functional experiments indicate that isotonitazene has high affinity for μ-opioid receptors and it is more potent than fentanyl in stimulating [^{35}S]GTPγS-binding. The single in vivo report from the original patent states that isotonitazene is 500 times more potent than morphine. Isotonitazene is also highly lipophilic. When identifying and analysing samples suspected of containing isotonitazene, laboratories need to consider aspects such as the high analytical sensitivity required to detect the sub-nanogram per millilitre amounts in biological samples; the fact that the n-propoxy isomer of isotonitazene will result in very similar mass spectrometry fragmentation patterns; and that many of the 2-benzylbenzimidazoles will have similar *O*-dealkylation biotransformation products.[1] The United Nations Office on Drugs and Crime Early Warning Advisory Tox-Portal and forensic toxicology reports from USA data[2] found that isotonitazene was associated with multiple deaths although, in most cases, it was administered in combination with other opioids and benzodiazepines. In a study quantifying isotonitazene and its metabolites, the concentrations of isotonitazene were lower than fentanyl and approximately similar to carfentanil.[1] Little information is available on online forums regarding usage and there are few trip reports. Therefore, most information on isotonitazene comes from the postmortem analyses and seizures reported to national and international monitoring organizations. Isotonitazene had only been identified for approximately 18 months at the time of writing this report, so patterns of manufacture, distribution and usage are only just emerging.

[1] Krotulski AJ, Papsun DM, Kacinko SL, Logan BK. Isotonitazene quantitation and metabolite discovery in authentic forensic casework. J Anal Toxicol. 2020;44:520–30. https://doi.org/10.1093/jat/bkaa016.

[2] US Drug Enforcement Administration – Drug and Chemical Evaluation Section Diversion Control Division. Temporary Schedule I Placement of N,N-Diethyl-2-[[4-(1-methylethoxy)phenyl]methyl]-5-nitro-1H-benzimidazole-1-ethanamine (isotonitazene) background, data, and analysis: three factor analysis pursuant to 21 U.S.C. 811(h)(3), 2020.

1. Substance identification

A. International Nonproprietary Name (INN)

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

B. Chemical Abstract Service (CAS) Registry Number

14188-81-9 free base

119276-00-5 hydrochloride salt

C. 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-1H-benzo[d]imidazol-1-yl)ethan-1-amine

N,N-diethyl-2-[2-(4-isopropoxybenzyl)-5-nitro-1H-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-1H-benzimidazole-1-ethanamine

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

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

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

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

1-(*N,N*-diethylamino-ethyl)-2-benzyl-5-nitro-benzimidazole

benzimidazole, 1-[2-(diethylamino)ethyl]-2-(p-isopropoxybenzyl)-5-nitro (6CI,7CI,8CI)

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

N,N-diéthyl-2-[2-(4-isopropoxybenzyl)-5-nitro-1H-benzimidazol-1-yl]éthanamine [French]

D. Trade names

Not applicable

E. Street names

Iso

Nitazene

Toni

F. Physical appearance

Yellow, brown or off-white powder

G. WHO review history

Isotonitazene has not previously been reviewed by the WHO Expert Committee on Drug Dependence.

2. Chemistry

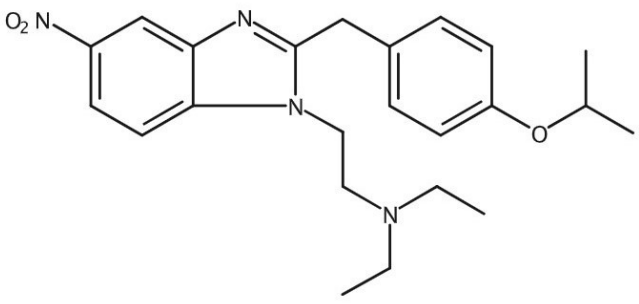
A. Chemical name

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

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

B. Chemical structure

Free base:



Molecular formula: $C_{23}H_{30}N_4O_3$

Molecular weight: 410.51

C. Stereoisomers

There are no isomers described for isotonitazene.

D. *Methods and ease of illicit manufacturing*

Isotonitazene is a 5-nitro-2-benzylbenzimidazole belonging to the 2-benzylbenzimidazole group of compounds originally developed as opioid analgesics. This group of compounds also includes the closely related homologues, etonitazene, metonitazene and clonitazene. The substitution at the paraposition of the benzyl moiety is the difference between isotonitazene, etonitazene and metonitazene: an isopropoxy group in isotonitazene, an ethoxy group in etonitazene, and a methoxy group in metonitazene. An ethereal isopropoxy group replacing the chloro halogen atom is the difference between isotonitazene and clonitazene. Protonitazene is the n-propoxy isomer of isotonitazene *(1)*.

As described in the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) technical report on isotonitazene, several methods have been developed for the synthesis of 2-benzylbenzimidazoles including isotonitazene *(2–4)*. In one method, 2-diethylaminoethylamine removes an activated chloro atom from 1-chloro-2,4-dinitrobenzene. Ammonium sulfide is used to selectively reduce the nitro function adjacent to the alkylamino moiety of the subsequent 2,4-dinitroaniline derivative. To obtain the final product – isotonitazene – the acquired orthophenylenediamine species is condensed with the imidate of 4-isopropoxyphenylacetic acid obtained from the corresponding cyanide. Acid-base extraction is used for purification, followed by conversion of the free base into its hydrochloride salt if desired. It is unclear which method is currently being used for the manufacture of the isotonitazene that has appeared on the illicit drug market *(1)*. One approach for synthesizing multiple 2-benzylbenzimidazole opioids is intended for the synthesis of etonitazene; it is apparently simpler and may be used for large-scale preparations *(5)*. Alternatively, isotonitazene may be produced from desethyletonitazene as described in the original patents *(6, 7)* and summarized in the EMCDDA report *(1)*. Finally, benzimidazoles were efficiently synthesized using three-component reactions of 2-haloanilines, aldehydes and NaN_3 in the presence of copper catalysts with commercially available starting materials and easy purification *(8)*.

E. Chemical properties

Melting point
172–173 °C

Boiling point
584.7 ± 45.0 °C at 760 mmHg

Solubility

Isotonitazene is predicted to be slightly soluble (1.0 g/L) at a temperature of 25 °C and pH 7. Due to its structural similarity to etonitazene, the free base could be expected to be sparingly soluble in water whereas the hydrochloride salt could be

expected to be more soluble. In a chemical characterization study, isotonitazene was solubilized in methanol for chromatographic analyses and assessment of biological activity, and in dimethyl sulfoxide for nuclear magnetic resonance (NMR) analysis *(9)*. No definitive solubility data on the salts of isotonitazene are available; however, since isotonitazene is similar to etonitazene, the salts are expected to be sufficiently water-soluble for administration of effective doses.

F. Identification and analysis

Analytical methods used for the characterization of isotonitazene in physical samples include high-performance liquid chromatography (HPLC), mass spectrometry, ultraviolet spectroscopy, infrared spectroscopy, Raman spectroscopy, ^{1}H NMR spectroscopy and ^{13}C NMR spectroscopy *(9–13)*. A recent study compared a sample of white homogeneous powder sold online in June 2019 as etonitazene to reference standard samples of isotonitazene using liquid chromatography time-of-flight mass spectrometry, gas chromatography-mass spectrometry (GC-MS), HPLC diode array detector, NMR spectroscopy and Fourier-transform infrared spectroscopy analysis. The sample sold as etonitazene was identified as isotonitazene *(9)*.

When identifying and analysing samples suspected of containing isotonitazene several factors need to be taken into consideration. One is that high analytical sensitivity is needed when testing because the concentrations of isotonitazene in biological samples are typically low to sub-nanogram per millilitre *(1)*. For example, in one forensic study, the average concentration in blood was 2.2 ± 2.1 ng/mL and was sometimes as low as 0.4 ng/mL *(13)*. A second factor is that GC-MS analysis of isotonitazene and its n-propoxy isomer, protonitazene, will result in very similar mass spectrometry fragmentation patterns *(9, 13)*.

3. Ease of convertibility into controlled substances

At the time of writing this report, no information was available on whether isotonitazene is converted into other controlled substances. However, the synthesis of isotonitazene is similar to etonitazene as well as clonitazene, as originally described in the patents *(2–4, 6, 7)*.

4. General pharmacology

A. Routes of administration and dosage

Based on information from online forums *(14–16)* and forensic reports *(13)*, the routes of administration of isotonitazene are vaping, intravenous, sublingual and intranasally via spray or insufflation. The doses reported on these online forums are quite variable ranging from 1–10 mg intravenously, sublingually or via vaping, and another report of 100–200 µg via nasal spray. In one report an isotonitazene user described feeling dependent on isotonitazene when consuming approximately 100 mg, sublingually or intravenously per day. This variability probably reflects the nature of Internet self-reports and the inability to verify that the substance consumed was really isotonitazene or how much of it was isotonitazene.

B. Pharmacokinetics

Isotonitazene was found to undergo de-alkylation to form *N*-desalkyl and *O*-desalkyl primary urinary metabolites based on finding *N*-desethyl-isotonitazene and *N*-desethyl-*O*-desalkyl-isotonitazene in five of six urine specimens tested by Krotulski et al. *(13)*. These authors reported that whereas reduction of the nitro group to form the metabolite 5-amino-isotonitazene occurred in only two urine samples, it occurred in 15 of the 18 blood samples. These authors also noted that *O*-dealkylation biotransformation products are believed to be common metabolites of the three benzimidazole compounds – isotonitazene, metonitazene and etonitazene. This suggests that these metabolites could prove useful in monitoring these types of opioids. However, the detection of metabolites alone would not help to determine whether isotonitazene, metonitazene or etonitazene was the drug consumed *(13)*.

Based on an ionization study of isotonitazene and other 2-benzylbenzimidazole analgesics, isotonitazene was observed to have apparent partition coefficients in the aqueous buffer (pH 7.4)-cyclohexane system greater than that of morphine. Differences were particularly extreme between morphine and isotonitazene. This suggested to the authors that isotonitazene, once administered, would be rapidly transported across lipid barriers to the site of action, i.e. it is a drug that penetrates the central nervous system rapidly *(17)*. As isotonitazene possesses a calculated log P of 4.85, it would be likely to be absorbed easily and to cross the blood–brain barrier *(1)*.

C. Pharmacodynamics

In radioligand binding assays, the dissociation constant (Ki) values for isotonitazene were: 0.323 ± 0.094 nM for the μ-opioid receptor (MOR) using [^{3}H] [D-Ala2, N-MePhe4, Gly-ol]-enkephalin (DAMGO), 271 ± 83 nM for the κ-opioid receptor (KOR) using [3H]U69,593 and 115 ± 24 nM for the δ-opioid receptor (DOR) using [^{3}H] [D-Pen2,D-Pen5]enkephalin (DPDPE). Therefore, isotonitazene selectively bound to μ-opioid receptors when [^{3}H]DAMGO was used as the radioligand *(18)*.

Isotonitazene was tested in an in vitro human embryonic kidney 293 T (HEK293 T) cell-reporter assay and found to activate MOR via interaction with β-arrestin2 with high potency median effective concentration (EC_{50}) of 11.1 nM and a high efficacy – 180% that of hydromorphone. This concentration-dependent response to stimulate MOR via β-arrestin2 was antagonized by naloxone (the authors did not publish these data) *(9)*. In another study, isotonitazene was tested in MOR activation assays in HEK293 T cells using β-arrestin2 as in the previous study, or G protein (mini-Gi) recruitment. In the β-arrestin2 recruitment assay, the EC_{50} for isotonitazene was 6.64 nM (2.84–15.0) with an E_{max} of 159% (140–178) and in the mini-Gi recruitment assay, the EC_{50} was 16.3 nM (10.6–25.5) with an E_{max} of 484% (444–525) relative to hydromorphone. In these studies, isotonitazene did not demonstrate any biased agonism *(19).*

When isotonitazene was evaluated in a [^{35}S]GTPγS functional assay using preparations of transfected Chinese hamster ovary cells expressing human δ- and κ-

opioid receptors and rat μ-opioid receptors, isotonitazene produced 114.6 ± 7.5%, 87.7 ± 3.5% and 106.5 ± 3.2% maximum stimulation with EC_{50} potencies of 548.6 ± 8.1 nM, 344 ± 99 nM and 0.381 ± 0.076 nM, respectively. Importantly, isotonitazene fully stimulated all three opioid receptors and was more potent than DAMGO (25.2 ± 2.3 nM) and fentanyl (27.9 + 4.2 nM) at μ-opioid receptors in this assay. In summary, isotonitazene was most effective and most potent at μ-opioid receptors *(18).*

The only in vivo pharmacology study on isotonitazene was reported in the original patent of the 2-benzylbenzimidazole group of opioids. In a mouse tail-flick assay, subcutaneously administered isotonitazene was 500 times more potent than morphine as an analgesic *(4, 6).*

5. Toxicology

No preclinical acute or chronic toxicology studies of isotonitazene have been reported.

6. Adverse reactions in humans

The clinical toxicological properties of isotonitazene have not been studied directly and there are few reports from user websites on the acute and chronic physical and psychological effects. Most of the information on adverse events associated with isotonitazene comes from postmortem, forensic toxicology studies. Toxicological case reports from 1 June 2019 to 23 September 2019 in the Midwest of the United States of America indicated that isotonitazene was involved in several fatalities. These included: one male aged 25–44 years with 0.9 ng/mL isotonitazene alone; one male aged 25–44 years with 1.7 ng/mL isotonitazene plus flualprazolam; two females with 1.0 and 4.4 ng/mL isotonitazene plus etizolam, one male aged >64 years and one male aged 25–44 years, both with 1.5–1.9 ng/mL isotonitazene plus etizolam; one male aged 45–64 years old with 4.4 ng/mL isotonitzene plus U-47700; and one person (sex not given) aged 45–64 years old with 0.4 ng/mL isotonitazene plus flualprazolam *(20)*. Isotonitazene has been associated with multiple deaths, although in most cases it was administered in combination with other opioids and benzodiazepines.

Biological samples suspected of containing isotonitazene, which included the cases reported to the United Nations Office on Drugs and Crime (UNODC) Early Warning Advisory (EWA)-Tox-Portal, were submitted from NMS Laboratories to the Center for Forensic Science Research and Education for forensic analysis, characterization and metabolite identification *(13)*. Isotonitazene was identified in 18 people who had died between August 2019 and January 2020 from these blood, urine and vitreous fluid samples. These deaths occurred in four states in the USA – Illinois, Indiana, Minnesota and Wisconsin. Twelve of the deceased were male and six were female with an average age of 41 years (range 24–66 years). Isotonitazene was the only opioid detected in half of the 18 samples, whereas, in the other half, other opioids such as fentanyl (6 samples), heroin (3 samples), tramadol (2 samples) and U-47700 (1 sample) were also detected. Some of the samples included other

benzodiazepine substances such as etizolam (6 samples) and flualprazolam (7 samples) as well as other compounds. The average concentration of isotonitazene detected in blood samples was 2.2 ± 2.1 ng/mL (range 0.4–9.5 ng/mL) *(13)*. The National Forensic Laboratory Information System (NFLIS) registered eight additional cases in which isotonitazene was involved: seven from Tennessee and one from California in 2019. The US DEA reported on another death, which occurred in January 2020 in Pennsylvania, in which isotonitazene was identified in a biological sample. However, the US DEA cautions that isotonitazene is most likely under-reported owing to the rapid appearance of the drug *(21)*.

Toxicovigilance Canada reported a death in Alberta Canada as early as March 2019 and two additional deaths in September and October 2019, although the specific role played by isotonitazene was not reported *(22)*. A death involving isotonitazene was reported in Germany but no details were available *(1).* The United Kingdom also reported the death of an individual with ng/mL concentrations of isotonitazene, butyrylfentanyl, despropionyl fentanyl (4-ANPP) and despropionyl fluorofentanyl. The extent to which isotonitazene contributed to this death is not known *(1).*

The case histories and autopsy findings relating to isotonitazene show similarities to those reported for use of traditional opioids, including heroin. There is also evidence of scars from injecting and puncture wounds, consistent with intravenous drug use. Signs associated with suspected opioid overdose such as pulmonary and/or cerebral oedema were often noted in the autopsy reports *(13).*

No formal studies on the psychological and behavioural effects of isotonitazene have been conducted. However, isotonitazene is likely to share the adverse effects commonly reported for other opioid analgesics such as incoordination, dizziness, drowsiness, mental confusion, sedation and profound intoxication *(23)*. Two users' reports on the online forums Drugs Forum and Reddit noted that the adverse effects were very dry eyes, constipation, joint "creakiness", sedation and mania at lower doses. However, very few self-reports about isotonitazene were available from Internet sites and, moreover, it is not possible to verify that the substance consumed was isotonitazene.

7. Dependence potential

A. Animal studies

The abuse liability and dependence potential of isotonitazene have not been studied in animals. However, studies of etonitazene, a closely related homologue to isotonitazene, revealed that discriminative stimulus *(24)*, self-administration *(25, 26)*, and tolerance and dependence *(27, 28)* are similar or greater than that of morphine or fentanyl. These studies suggest that isotonitazene, a member of the 2-benzylbenzimidazole group of opioids like etonitazene, would have an abuse liability and dependence-producing potential in animals.

B. Human studies

The abuse liability and dependence potential of isotonitazene have not been studied in humans. Only one online forum thread described dependence relating to isotonitazene use. A person who reported frequent use of isotonitazene described symptoms of physical dependence after taking up to 100 mg isotonitazene intravenously or sublingually for up to 5 months. The symptoms of dependence, according to two users on Reddit, included withdrawal symptoms of fever, dizziness, flu-like feelings, blackouts, anxiety and panic attacks. Few self-reports from Internet sites are available and it is not possible to verify that the substance consumed was isotonitazene.

8. Therapeutic applications and extent of therapeutic use and epidemiology of medical use

There are currently no therapeutic applications or recorded medical uses of isotonitazene.

9. Listing on the WHO Model List of Essential Medicines

Isotonitazene is not listed on the WHO Model List of Essential Medicines.

10. Marketing authorizations (as a medicinal product)

No evidence is available that isotonitazene is being considered as a medicinal product. Furthermore, isotonitazene has never been granted a marketing authorization as a medicinal product for human or veterinary use, has not been the subject of an application for a marketing authorization as a medicinal product for human or veterinary use.

11. Industrial use

No potential industrial use was identified for isotonitazene besides as an analytical reference standard for scientific research and forensic applications. Isotonitazene is available for purchase from various chemical companies and is available in wholesale amounts and in consumer amounts.

12. Nonmedical use, abuse and dependence

No formal epidemiology reports have been published on the prevalence, abuse or dependence potential of isotonitazene. Only the toxicology case reports described above, and the seizures described below indicate patterns or potential patterns of nonmedical use. However, the population likely to abuse isotonitazene appears to be the same as that using heroin, prescription opioid analgesics and other synthetic opioid substances *(21).* This is evidenced by the other types of drugs typically identified together with isotonitazene in biological samples obtained from fatal overdose cases. Furthermore, a report from BlueLight described isotonitazene as having the “potency of fentanyl + duration of heroin, with actual euphoria to go with it” and another from Drugs Forum stated “This is my favorite drug I’ve ever done. A heavy rush similar to fentanyl, but with the euphoria of quality heroin. Extremely powerful, this dose would kill or at least incapacitate the average street

user". There are few descriptions of isotonitazene on the readily available online forums *(14–16).* One user reported use for 5 months and another for 2 months on Reddit. Both described adverse effects such as extremely dry eyes, dehydration and constipation. The reported symptoms of dependence included withdrawal symptoms of fever, dizziness, flu-like feelings, blackouts, anxiety and panic attacks.

13. Nature and magnitude of public health problems related to misuse, abuse and dependence

Toxicological case reports indicating that isotonitazene was involved in fatalities in midwestern USA *(20)* have been discussed in section 6. In the 18 toxicological cases analysed at the Center for Forensic Science Research and Education (including the eight cases listed above) (see section 6), some of those who died were regular heroin and opioid users, suggesting that isotonitazene may be a substitute for heroin or other opioids *(13, 21)*. Indeed, the US DEA-VA reported the seizure of a powder mixture of isotonitazene, heroin and bromazolam *(21)*. For the purpose of experimental analysis, "etonitazene" was purchased through an online supplier but was actually isotonitazene *(9)*.

There are no reports of impaired driving or harm to others. Isotonitazene is apparently the first of the 2-benzylbenzimidazole opioids to be identified on the current illicit drug market. Its novelty could increase the potential for accidental overdose or life-threatening poisoning if an individual is unfamiliar with how to dose the new substance *(21)*. The risk is greater if isotonitazene is sold under another name or mixed with other drugs *(21).*

14. Licit production, consumption and international trade

The only licit production is as an analytical reference material classified as an opioid intended for research and forensic applications. It is synthesized by various chemical companies and is available in wholesale and consumer quantities. A labelled version, isotonitazene-d7 is also available for purchase through the same vendors for use with GC-MS or LC-MS methods for research and forensic purposes.

15. Illicit manufacture and traffic and related information

Isotonitazene is sold online as a powder, a ready-to-use nasal spray or in counterfeit pills *(1).* The size and scale of the manufacture and trafficking operations are not known. A Reddit user announced that a Chinese vendor would be preparing a new batch for distribution after the Chinese New Year in 2019, as reported in the online Filter Magazine *(29)*. The first appearance of isotonitazene appears to have been in March 2019 in Alberta, Canada *(22)*. In February and March 2020, law enforcement officials found isotonitazene in the form of a triangular white tablet with an "M" logo on one side and an "8" logo on the other side and as a blue tablet in counterfeit Dilaudid pills *(30)*.

In Europe, isotonitazene appeared in a seizure in Estonia in April 2019.

At the time of the technical report on isotonitazene published by the EMCDDA, isotonitazene had also been identified in Belgium, Germany, Latvia, Sweden and the

United Kingdom *(1)*. Seizures has been reported in Estonia (*n* = 17, isotonitazene in powder form), Germany (*n* = 2, 4.5 g isotonitazene in liquid form, one sample with a synthetic cannabinoid) and Latvia (*n* = 4, isotonitazene in powder form, one sample with fentanyl). A total of 109.6 g of isotonitazene powder was seized between April 2019 and January 2020. Other seizures in which isotonitazene was identified were reported by Sweden in a customs seizure (48.8 g), by Belgium as a collected sample, and in the United Kingdom in biological samples following a death *(1).*

In the United States, isotonitazene in powder form has been identified as a single substance or in combination with other substances. According to the NFLIS database, there had been eight cases in which isotonitazene was identified in the United States as of 5 March 2020. They occurred in 2019 in two states: seven cases in Tennessee and one in California. A seizure of 1.6 g of isotonitazene in California was reported in April 2019. In addition, Wisconsin State Crime Laboratories identified isotonitazene, bromazolam and heroin in a mixed seized powder. In the state of Iowa, isotonitazene was found at the scene of investigation of a death *(21).*

Although isotonitazene has been identified in Canada, Europe and the United States, the extent of the illicit market is unknown. A statement from a user on Reddit claimed that the isotonitazene market may be limited. Anecdotal reports from February 2020 suggest that the presence of isotonitazene, at least in the United States, is not likely to increase because some manufacturers have allegedly discontinued production of isotonitazene and have moved on to the production of other analogues *(29)*.

16. Current international controls and their impact

Isotonitazene is not controlled under the Convention on Psychotropic Substances of 1971 or under Schedule I of the United Nations Single Convention on Narcotic Drugs of 1961 as amended by the 1972 Protocol *(31, 32).* However, other drugs belonging to the same class – etonitazene and clonitazene – are controlled under the United Nations Single Convention on Narcotic Drugs of 1961 *(31, 33).*

17. Current and past national controls

The US DEA placed a temporary order to schedule isotonitazene, including its isomers, esters, ethers, salts and salts of isomers, esters and ethers whenever the existence of such isomers, esters, ethers and salts is possible, in schedule I of the Controlled Substances Act on 18 June 2020 *(34)*.

The United Kingdom controls isotonitazene via the legislation on new psychoactive substances. Estonia, Latvia, Poland and Sweden have placed isotonitazene under restrictive measures. Lithuania and Norway control isotonitazene through legislation on medicines. It is not known whether isotonitazene is controlled in China *(1)*.

Isotonitazene is not subject to restrictive measures at national level by the 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. Isotonitazene is not subject to restrictive measures at national level in Turkey *(1).*

18. Other medical and scientific matters relevant for a recommendation on the scheduling of the substance

Very limited pharmacological information is available on isotonitazene, which could increase the risk of harmful adverse events. There is no information on the social harm that may be caused by isotonitazene. It is likely, however, that the risks may be similar to those associated with the use of established opioids, especially etonitazene, metonitazene and clonitazene, which are derived from the same class of benzimidazole compounds.

References

1. EMCDDA (European Monitoring Centre for Drugs and Drug Addiction). EMCDDA technical report on the new psychoactive substance N,N-diethyl-2-[[4-(1-methylethoxy)phenyl]methyl]-5-nitro-1Hbenzimidazole-1-ethanamine (isotonitazene). Lisbon: EMCDDA; 2020 (https://www.emcdda.europa.eu/system/files/publications/13108/EMCDDA%20technical%20report%20on%20isotonitazene.pdf).
2. Hunger A, Kebrle J, Ross, A, Hoffmann K. 'Synthese basisch substituerter, analgetisch wirksamer Benzimidazol-Derivate', Experientia. 1957;13:400–401.
3. Hunger A, Kebrle J, Ross, A, Hoffmann K. Benzimidazol-Derivate und verwandte Heterocyclen. II. Synthese von 1-Aminoalkyl-2-benzyl-benzimidazolen. Helvetica Chimica Acta. 1960;43:800–809.
4. Hunger A, Kebrle J, Ross, A, Hoffmann K. Benzimidazole-Derivate und verwandte Heterocyclen III. Synthese von 1-Aminoalkyl-2-benzyl-nitro-benzimidazolen. Helvetica Chimica Acta. 1960:43:1032–46,.
5. Carroll FI, Coleman MC. Etonitazene. An improved synthesis. J Med Chem. 1975;18:318–20.
6. Hoffmann K, Hunger A, Kebrle J, Rossi A. Analgetically active benzimidazoles. 1959. DE patent 1057123B.
7. Hoffmann K, Hunger A, Rossi A. Benzimidazoles, 1960. United States Patent Office, 2, 935,514. https://patents.google.com/patent/US2935514A/en.
8. Kim Y, Kumar MR, Park N, Heo Y, Lee S. Copper-catalyzed, one-pot, three-component synthesis of benzimidazoles by condensation and C-N bond formation. J Org Chem. 2011;76:9577–83.
9. Blanckaert P, Cannaert A, Van Uytfanghe K, Hulpia F, Deconinck E, Van Calenbergh S, Stove C. Report on a novel emerging class of highly potent benzimidazole NPS opioids: Chemical and in vitro functional characterization of isotonitazene. Drug Test Anal. 2019, https://doi.org/10.1002/dta.2738

10. NPS Discovery. Isotonitazene. Center for Forensic Science Research and Education (CFSRE), 2019 (https://www.npsdiscovery.org/wpcontent/uploads/2019/11/Isotonitazene_112119_ToxicologyAnalyticalReport.pdf).

11. ADEBAR Analytical Report. Isotonitazene. Case report: EDND-CR-2020-131, Ausbau analytischer Datenbanken, Erhebung und bundesweite Bereitstellung von analytischen Daten, pharmakologischen Daten, Verfahrensvorschriften und Referenzmaterialien für neu auf dem Drogenmarkt auftretende Stoffe. 2020. (http://ednd2.europa.eu/ednd/substanceProfiles/1054, accessed 23 August 2020).

12. Cayman Chemical. Isotonitazene, 2020 (https://www.caymanchem.com/product/27255).

13. Krotulski AJ, Papsun DM, Kacinko SL, Logan BK. Isotonitazene quantitation and metabolite discovery in authentic forensic casework. J Anal Toxicol. 2020; 44:520–30. https://doi.org/10.1093/jat/bkaa016

14. BlueLight. Isotonitazene. [website] (https://www.bluelight.org/xf/search/743595/?q=isotonitazene&o=date, accessed 23 August 2020).

15. Drugs Forum. Trip report: isotonitazene (5mg iv) + dexedrine (30mg oral) [website] (https://drugs-forum.com/threads/trip-report-isotonitazene-5mg-iv-dexedrine-30mg-oral.353880/, accessed 23 August 2020).

16. Reddit. Symptoms of chronic isotonitazene use/abuse [website] (https://www.reddit.com/r/Opioid_RCs/comments/f2f5s2/symptoms_of_chronic_isotonitazene_useabuse/, accessed 23 August, 2020).

17. Casy AF, Wright J. Ionisation constants and partition coefficients of some analgesically active 2-benzylbenzimidazole derivatives and related compounds. J Pharm Pharmacol. 18;1966:677-83.

18. US Drug Enforcement Administration–Veterans Affairs (DEA-VA) Interagency Agreement. Isotonitiazene. N,N-diethyl-2-[[4-(1-methylethoxy)phenyl]methyl]-5-nitro-1H-benzimidazole-1-ethanamine. Binding and functional activity at delta, kappa and mu opioid receptors. in vitro receptor and transporter assays for abuse liability testing for the DEA by the VA. Springfield (VA): US DEA; 2019.

19. Vandeputte MM, Cannaert A, Stove CP. In vitro functional characterization of a panel of non-fentanyl opioid new psychoactive substances. Arch Toxicol. Published online, 31 July 2020. doi.org/10.1007/s00204-020-02855-7.

20. UNODC Early Warning Advisory (EWA) Tox-Portal. Isotonitazene (https://www.unodc.org/tox/#/admin/search/detailed/%7B%22textQuery%22:%22isotonitazene%22%7D, accessed 27 July 2020).

21. US Drug Enforcement Administration – Drug and Chemical Evaluation Section Diversion Control Division. Temporary Schedule I Placement of N,N-Diethyl-2-[[4-(1-methylethoxy)phenyl]methyl]-5-nitro-1H-benzimidazole-1-ethanamine (Isotonitazene) Background, data, and analysis: three factor analysis pursuant to 21 U.S.C. 811(h)(3). Springfield (VA): US DEA; 2020.

22. Toxicovigilance Canada. Isotonitazene: Newly identified opioid in Alberta. 11 October 2019.

23. Ujváry I. Technical review of new synthetic opioids identified on the European drug market. Part 1. Benzimidazole opioids. Lisbon: European Monitoring Centre for Drugs and Drug Addiction; 2020 (EMCDDA Contract Code CT.20.SAS.00017.1.0).

24. Zhang L, Walker EA, Sutherland J 2nd, Young AM. Discriminative stimulus effects of two doses of fentanyl in rats: pharmacological selectivity and effect of training dose on agonist and antagonist effects of mu opioids. Psychopharmacol (Berl). 2000;148:136–45. doi: 10.1007/s002130050035.

25. Achat-Mendes C, Valdez GR, Platt DM, Rowlett JK, Spealman RD. Intravenous self-administration of etonitazene alone and combined with cocaine in rhesus monkeys: comparison with heroin and antagonism by naltrexone and naloxonazine. Psychopharmacology (Berl). 2009;204:489–98. doi: 10.1007/s00213-009-1480-0.

26. Ginsburg BC, Lamb RJ. Frustration stress (unexpected loss of alternative reinforcement) increases opioid self-administration in a model of recovery. Drug Alcohol Depend. 2018:182;33–39. doi: 10.1016/j.drugalcdep.2017.09.016.

27. Walker EA, Young AM. Differential tolerance to antinociceptive effects of mu opioids during repeated treatment with etonitazene, morphine or buprenorphine in rats. Psychopharmacol (Berl). 2001;154:131–42. doi: 10.1007/s002130000620.

28. Tang AH. The etonitazene-dependent rhesus monkey as a model to study narcotic agonist and antagonist activities. NIDA Res Monogr. 1982;41:200-7.

29. Blanchard SK. Why isotonitazene is not yet the “new fentanyl”. Filter Magazine, 21 May 2020 (https://filtermag.org/isotonitezene/).

30. Halifax Police, Police warn public of potent synthetic opioid found in Halifax, 2020 (https://www.halifax.ca/home/news/police-warn-public-potent-synthetic-opioid-found-halifax).

31. UNODC (United Nations Office on Drugs and Crime). Schedules of the Single Convention on Narcotic Drugs of 1961 as amended by the 1972 Protocol, as at 24 May 2019 (https://www.unodc.org/unodc/en/commissions/CND/conventions.html).

32. UNODC (United Nations Office on Drugs and Crime). Schedules of the Convention on Psychotropic Substances of 1971, as at 24 May 2019 (https://www.unodc.org/unodc/en/commissions/CND/conventions.html).

33. WHO Expert Committee on Addiction-Producing Drugs: eleventh Report. Geneva: World Health Organization; 1961 (WHO Technical Report Series, No. 211; https://apps.who.int/iris/handle/10665/40481).

34. US Drug Enforcement Administration – Department of Justice. Schedules of Controlled Substances: Temporary Placement of Isotonitazene into Schedule I. Federal Register Volume 85, Number 118 (Thursday, June 18, 2020)] [Proposed Rules] [Pages 36819-36823], Springfield (VA): US DEA; 2020.

.

Annex 1. Report on WHO Questionnaires for Review of Psychoactive Substances for the 43rd ECDD: evaluation of Isotonitazene

Data were obtained from 105 Member States (19 African Region, 13 Eastern Mediterranean Region, 40 European Region, 16 Region of the Americas, seven South-East Asia Region and 10 Western Pacific Region) for the WHO Questionnaires for the Review of Psychoactive Substances. The total number of countries opting out of participation in the questionnaire is 13 (three African Region, two Eastern Mediterranean Region, two European Region, three Region of the Americas, one South-East Asia Region and two Western Pacific Region), leaving 92 active countries. Of these, 29 countries had information on the substance (Table 1).

Table 1. Numbers of countries providing information on Isotonitazene

Region	Number of countries without information	Number of countries with information on substance
African Region	15	1
Eastern Mediterranean Region	8	3
European Region	22	16
Region of the Americas	9	4
South-East Asia Region	5	1
Western Pacific Region	4	4
Total 92	**63**	**29**

LEGITIMATE USE

One country (Eastern Mediterranean Region) reported approved human medical products and veterinary products containing Isotonitazene.

One country (Region of the Americas) reported Isotonitazene being currently used in medical or scientific research (excluding use as an analytical standard), specifically in cell line studies (binding/functional assays) and animal studies.

One country (Region of the Americas) reported that Isotonitazene being used in industrial or other non-medical or non-scientific use.

No countries reported approved therapeutic indications for Isotonitazene.

EPIDEMIOLOGY OF NON-MEDICAL/NON-SCIENTIFIC USE – USE FOR PSYCHOACTIVE PURPOSES OR RECREATIONAL DRUG USE

Eight countries (five European Region, two Region of the Americas, one Western Pacific Region) reported that Isotonitazene is being misused or abused for its psychoactive properties/recreational use.

The most common known route of administration reported was oral, followed by injection (Table 2).

Table 2. Common routes of administration

Route of administration	Number of countries
Oral	3
Injection	2
Inhalation	0
Sniffing	1
Smoking	1
Don't know	18

The most common known formulation of Isotonitazene reported was powder (Table 3).

Table 3. Common formulations reported by countries

Formulation	Number of countries
Powder	4
Tablets	1
Liquid for oral use	1
Solution for injection	0
Don't know	16

Six countries reported the level of negative health impact due to Isotonitazene's non-medical consumption as "serious" or "substantial" (Table 4).

Table 4. Level of negative health impact

Serious	Substantial	Negligible	Don't know
5	1	8	14

One country (Region of the Americas) noted numerous seizures of Isotonitazene and commented that, since Isotonitazene is more potent than fentanyl, it may lead to fatal overdoses. Another country (European Region) noted that, since there is no reporting obligation by hospitals, poison centres, etc., there may be unreported cases of negative health impacts. One country (Region of the Americas) stated that Isotonitazene resulted in adverse health effects including death and that Isotonitazene has been positively identified in numerous non-fatal and fatal cases.

Two countries (one European Region, one Region of the Americas) reported emergency room admissions related to the non-medical use of Isotonitazene.

One country (Region of the Americas) listed the adverse effects that patients have presented with at emergency rooms/departments as opioid intoxication and central nervous system depression.

One country (European Region) reported Isotonitazene users presenting for drug dependence treatment.

Regarding mortality, four countries (two European Region, two Region of the Americas) reported deaths involving Isotonitazene:

- one fatal case where it was unknown whether other substances were involved (2019)
- three fatal cases where only total given (2020)
- 67 fatal cases where other substances were involved (2020).

STATUS OF NATIONAL CONTROL AND POTENTIAL IMPACT OF INTERNATIONAL CONTROL

Eight countries (five European Region, two Region of the Americas, one Western Pacific Region) responded that Isotonitazene is currently controlled under national legislation to regulate its availability. One country (European Region) stated, "The substance Isotonitazene has been evaluated for scientific and healthy aspects and is under approval process by authorities for the inclusion in Table I of Narcotics and Psychotropic Substances". Another country (European Region) noted that, "This substance is controlled as a narcotic drug from 21st September 2020 onwards".

Table 5 shows the main reported activities involving Isotonitazene.

Table 5. Reported illicit activities involving Isotonitazene

Activities	Number of countries
Smuggling from other countries	1
Manufacture of substance by chemical synthesis	0
Manufacture of substance by extraction from other products	0
Production of consumer products containing the substance	0
Trafficking	3
Diversion from legal supply chain	0
Internet sales – seller or website located in country	1
Internet sales – from abroad to buyers in country	3
Internet sales – other, or location of sellers and website unknown	3
Direct sales to people who use the substance	1
Don't know	16

In addition to the above, countries added:

- trafficking through postal services
- assumed dark web sale from unknown origin.

Three countries (two European Regions, one Region of the Americas) reported seizures (Table 6).

Table 6. Reported seizures of Isotonitazene

Year	Seizures
2020[1]	41
2019	35
2018	0
Total	**76**

[1]The country reporting these 41 seizures stated that the data are for both 2020 and 2019.

Twenty-two countries have the forensic laboratory capacity to analyse Isotonitazene.

One country (European Region) noted that, "Forensic laboratories have the capacity to analyse Isotonitazene if reference material is available".