World Health Organization

Critical Review Report:

N-pyrrolidino isotonitazene

(Isotonitazepyne)

Agenda item 3.3.1

Expert Committee on Drug Dependence

Forty-eighth Meeting

Geneva, 20-22 October 2025

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

N-pyrrolidino isotonitazene (Isotonitazepyne) belongs to the 2-benzylbenzimidazole class of opioids, commonly known as "nitazenes." It was first synthesized in the 1950s during a medication development effort by CIBA Aktiengesellschaft in Switzerland and appeared in Australia and the United States as a novel psychoactive substance in 2024. *N*-pyrrolidino isotonitazene binds with high affinity to the μ-opioid receptor (ED50 = 0.25 nM) and activates it with full efficacy at a potency that is at least five-fold higher than fentanyl. In vivo, it has potent analgesic effects in mice that last up to 45 minutes and are reversible with the μ-opioid receptor antagonist naltrexone. Further, it produces dose-dependent morphine-like discriminative stimulus effects in rats, suggesting that it would have subjective effects similar to those of morphine in humans. People who use *N*-pyrrolidino isotonitazene report that the compound produces euphoria and helps to prevent withdrawal in individuals dependent upon opioids. In humans, anecdotal evidence suggests that *N*-pyrrolidino isotonitazene is administered via several routes, including intravenously, vaping, and insufflation of a nasal spray. While no studies have been published detailing the pharmacokinetics of *N*-pyrrolidino isotonitazene, the metabolite 4-OH-nitazepyne has been identified as shared by all pyrrolidino nitazene derivatives and can serve as a marker of this subtype. Information on the physical and/or psychological effects specifically associated with the use of *N*-pyrrolidino isotonitazene (versus nitazenes as a general class) was not available in published or grey literature. Although the substance was present in one post-mortem sample in Finland, the degree to which *N*-pyrrolidino isotonitazene contributed to death was unclear, as a benzodiazepine and another opioid were also found. *N*-pyrrolidino isotonitazene has been identified in toxicology samples in Australia, the Netherlands, and the US during 2024 and 2025. Its effects in these cases were not reported. Laboratory analysis of drug samples has shown the presence of *N*-pyrrolidino isotonitazene in falsified prescription opioid tablets, in substances advertised as another opioid, and in mixtures with other non-opioid novel psychoactive substances. At least seven countries have reported detection of *N*-pyrrolidino isotonitazene within their borders. *N*-pyrrolidino isotonitazene is not currently under international control, but it is controlled nationally through national regulations controlling nitazenes as a chemical class in Canada and the United Kingdom. In 2025, an order for the temporary placement of *N*-pyrrolidino isotonitazene under Schedule 1 of the US Controlled Substances Act was published.

1. Substance identification

A. International Nonproprietary Name (INN)

Not available.

B. Chemical Abstract Service (CAS) Registry Number

3053113-12-2

C. Other Chemical Names

Isotonitazepyne
5-nitro-2-[(4-propan-2-yloxyphenyl)methyl]-1-(2-pyrrolidin-1-ylethyl)benzimidazole

D. Trade Names

N-pyrrolidino isotonitazene is sold under its own name or isotonitazepyne (Cayman Chemical, 2025)

E. Street Names

N-pyrrolidino isotonitazene is indicated under its own name or as isotonitazepyne (Curtis et al., 2025).

F. Physical Appearance

N-pyrrolidino isotonitazene citrate, as a synthetic standard appears as a crystalline solid (Cayman Chemical, 2025). In September 2024, the substance was identified in falsified pharmaceuticals, specifically in unmarked, round, mottled yellow tablets submitted to the Australian drug checking service CanTEST (CanTEST Health and Drug Checking Service) and expected to contain oxycodone. The latter was not detected by chemical analysis (Curtis et al, 2025).

2. Chemistry

A. Chemical Name

IUPAC Name:
5-nitro-2-[4-(2-propoxy)benzyl]-1-[2-(pyrrolidin-1-yl)ethyl]-1*H*-benzo[*d*]imidazole

CA Index Name:
1*H*-Benzimidazole, 2-[[4-(1-methylethoxy)phenyl]methyl]-5-nitro-1-[2-(1-pyrrolidinyl)ethyl]- (ACI)

Canonical SMILES
O=N(=O)C=1C=CC2=C(N=C(N2CCN3CCCC3)CC4=CC=C(OC(C)C)C=C4)C1

InChI

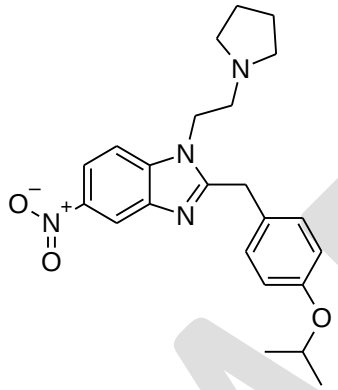
InChI=1S/C23H28N4O3/c1-17(2)30-20-8-5-18(6-9-20)15-23-24-21-16-19(27(28)29)7-10-22(21)26(23)14-13-25-11-3-4-12-25/h5-10,16-17H,3-4,11-15H2,1-2H3

InChI Key

VRKDSDBBRNHHCR-UHFFFAOYSA-N

B. Chemical Structure

Free base:



Molecular Formula: $C_{23}H_{28}N_4O_3$
Molecular Weight: 408.49 g/mol

C. Stereoisomers

There are no stereoisomers described for *N*-pyrrolidino isotonitazene.

D. *Methods and Ease of Illicit Manufacturing*

N-pyrrolidino isotonitazene belongs to the 2-benzylbenzimidazole class of opioids, commonly known as "nitazenes." It is a positional isomer of *N*-pyrrolidino protonitazene, but features an isopropoxy group instead of a propoxy group at the *para* position of the benzyl ring (Curtis et al., 2025; De Vrieze et al., 2024).

Although the synthesis of this specific nitazene has not been documented in the literature, the methods established for the 5-nitro-2-benzylbenzimidazole analogues, such as etonitazene, can be applied with appropriate modifications to the reagents to prepare *N*-pyrrolidino isotonitazene (Vandeputte et al., 2021; Caprari et al., 2025).

E. Chemical Properties

Melting point

No information could be identified.

Boiling point

No information could be identified.

Solubility

N-pyrrolidino isotonitazene as citrate salt is soluble in dimethyl sulfoxide (DMSO) and in dimethylformamide (DMF) at 10 mg/mL. It is soluble at 1 mg/mL in phosphate-buffered saline (PBS) (pH 7.2) (Cayman Chemical, 2025).

F. Identification and Analysis

N-pyrrolidino isotonitazene citrate is available as a reference material from commercial suppliers and used for routine methods of analysis associated with forensic and clinical investigation (Cayman Chemical, 2025).

N-pyrrolidino isotonitazene was first identified in September 2024 by drug checking services in Australia in an expected oxycodone sample presented by a member of the public and subsequently characterized via Fourier Transform Infrared (FTIR), Ultraperformance Liquid Chromatography-Photo-Diode Array (UPLC-PDA), High Resolution Ultraperformance Liquid Chromatography–Electrospray Ionisation–Tandem Mass Spectrometry (UPLC-ESI-MS/MS), gas chromatography-electron ionisation-mass spectrometry (GC-EI-MS), and proton and carbon nuclear magnetic resonance (^{1}H NMR, ^{13}CNMR) (Curtis et al., 2025).

N-pyrrolidino isotonitazene was detected in blood by liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) (Walton et al., 2024).

3. Ease of Convertibility Into Controlled Substances

It is not known from the literature whether *N*-pyrrolidino isotonitazene can be converted into a controlled substance.

4. General Pharmacology

A. Routes of administration and dosage

Data on the route of administration for *N*-pyrrolidino isotonitazene specifically were sparse. Anecdotally, preparation for vaping, smoking, intranasal spray, or intravenous use was reported by people who have obtained powder containing the substance (Reddit, 2025). Oral use was also reported. However, this information may not be representative of the most common route(s) of administration due to self-selection of whether or not to post to online forums. Dosage information was also sparse. One person who used *N*-pyrrolidino isotonitazene advised the use of insufflated doses in the "low double digit μg" for naïve users and restriction to "well below" mg doses for tolerant individuals (Reddit, 2025).

B. Pharmacokinetics

While no studies have been published detailing the pharmacokinetics of *N*-pyrrolidino isotonitazene, the metabolite 4-OH-nitazepyne has been identified as shared by all pyrrolidino nitazene derivatives and can serve as a marker of this subtype (Kriikku et al.,

2025). No studies were identified on the absorption, distribution, or elimination of *N*-pyrrolidino isotonitazene.

C. Pharmacodynamics

N-pyrrolidino isotonitazene binds with high affinity to the μ opioid receptor (MOR) [EC50 = 0.25 nM (± 0.58 nM, standard error of the mean) (Kozell et al., 2024) and Ki = 0.2392 nM (± 0.0072 nM, standard error of the mean) (Janowsky, 2023)]. By comparison, fentanyl's affinity for MOR was 4- to 5-fold less: EC50 = 1.255 nM (± 0.84 nM, standard error of the mean) (Kozell et al., 2024) and Ki = 0.96 nM (± 0.14 nM, standard error of the mean) (Janowsky, 2023). Both substances showed selective binding for MOR, with considerably lower affinity for kappa and delta opioid receptors. Further, *N*-pyrrolidino isotonitazene was a full, potent agonist at MOR, as measured by activation of [^{35}S]GTPγS [EC50 = 0.574 nM (± 0.012 nM, standard error of the mean) and Emax = 98% (± 1.2%, standard error of the mean)] (Kozell et al., 2024) and by inhibition of forskolin-stimulated cyclic adenosine monophosphate [EC50 = 0.04 nM (95% confidence interval: 0.02; 0.06 nM) and Emax = 105% (95% confidence interval: 99; 111 nM)] (De Vrieze et al., 2024). At the MOR, *N*-pyrrolidino isotonitazene also enhanced β-arrestin 2 recruitment, with an EC50 value of 0.288 nM (95% confidence interval: 0.20; 0.41 nM) (De Vrieze et al., 2024).

In mice, *N*-pyrrolidino isotonitazene had potent analgesic effects in a warm-water tail-flick test (ED_{50} = 0.001 mg/kg subcutaneously, standard error of the mean: ± 0.055 mg/kg) (Gatch et al., 2025). In this assay, it was nine-fold more potent than fentanyl in mice (fentanyl ED_{50} = 0.009 mg/kg). The peak effect occurred at a dose of 0.01 mg/kg, and percentage of maximum possible antinociceptive effect remained above 80% for 45 min after administration. Further, this effect was reversible by naltrexone, suggesting mediation by the MOR.

5. Toxicology

No studies of the preclinical toxicology of *N*-pyrrolidino isotonitazene were found.

6. Adverse Reactions in Humans

Information on the physical and/or psychological effects specifically associated with the use of *N*-pyrrolidino isotonitazene was not identified in published literature. Although the substance was present in one post-mortem sample in Finland in 2025, other substances were also identified, including a benzodiazepine and another opioid; hence, the degree to which *N*-pyrrolidino isotonitazene contributed to the death was unclear (Kriikku et al., 2025). *N*-pyrrolidino isotonitazene concentrations in the femoral blood and urine samples of the decedent were 1.4 and 2.6 μg/L, respectively. In Australia, *N*-pyrrolidino isotonitazene was reported to produce "significant nonfatal drug-related harm" in an individual who submitted pills containing the substance to a national drug-testing service (Curtis et al., 2025). The nature of this harm was not specified. Respiratory and cardiac arrest resulting from consumption of *N*-pyrrolidino isotonitazene were reported in a case from the Netherlands, with full resolution of symptoms after administration of naloxone (Balster et al., 2025). In the US, *N*-pyrrolidino isotonitazene was detected in one toxicology

case in 2024 and in four toxicology samples in the first two quarters of 2025 (Center for Forensic Science Research and Education, 2025) (see Section 13). The effects of the substance were not reported.

While there are only a few online descriptions of the effects of *N*-pyrrolidino isotonitazene from people who have used it, the predominant effects mentioned were euphoria and postponement of withdrawal (Reddit, 2025). Posts on online forums on self-reported experience of use of *N*-pyrrolidino isotonitazene should be considered anecdotal, as no analytical confirmation of sole use was obtained.

7. Dependence Potential

A. Animal Studies

No information was found.

B. Human Studies

No information was found.

8. Abuse Potential

A. Animal Studies

Drug discrimination is a pharmacologically selective animal model of the subjective effects of psychoactive drugs in humans. In a study with male Sprague-Dawley rats trained to discriminate morphine (3.2 mg/kg subcutaneously) from vehicle in a standard two-lever procedure, *N*-pyrrolidino isotonitazene caused full dose-dependent substitution for the morphine training dose, with an ED_{50} of 0.00045 mg/kg subcutaneously (standard error of the mean: ± 0.061 mg/kg) (Gatch et al., 2025). The maximum response on the morphine-associated lever of 88.9% (± 9%) was obtained at a dose of 0.001 mg/kg. The potency ratio (ED_{50} test compound/ ED_{50} reference compound) of *N*-pyrrolidino isotonitazene was 1800 when compared with morphine and 20 when compared with fentanyl. The three compounds had approximately equal efficacy in the procedure. The morphine-like discriminative stimulus effects of *N*-pyrrolidino isotonitazene (0.001 mg/kg) were reversed by naltrexone (1 mg/kg). These results suggest that *N*-pyrrolidino isotonitazene has subjective effects in humans similar to those of morphine.

B. Human Studies

No information was found.

9. Therapeutic Applications and Extent of Therapeutic Use and Epidemiology of Medical Use

There are no known therapeutic uses for *N*-pyrrolidino isotonitazene.

10. Listing on the WHO Model List of Essential Medicines

N-pyrrolidino isotonitazene is not listed on the 23rd WHO Model List of Essential Medicines or on the 9th WHO Model List of Essential Medicines for Children.

11. Marketing Authorizations (as a Medicinal Product)

N-pyrrolidino isotonitazene has no known marketing authorizations.

12. Industrial Use

N-pyrrolidino isotonitazene has no known industrial use.

13. Non-Medical Use, Abuse, and Dependence

N-pyrrolidino isotonitazene was first synthesized in the 1950s during the development of a medication by CIBA Aktiengesellschaft in Switzerland; however, it was not submitted for regulatory approval or brought to the legal drug market. *N*-pyrrolidino isotonitazene was first identified in the fall of 2024 in Australia and, shortly thereafter, in the US (Curtis et al., 2025; Walton et al., 2024). Reports on online forums by people who use drugs provide evidence that *N*-pyrrolidino isotonitazene has been used intentionally for its intoxicating effects (see section 6). To date, the presence of this substance has been reported in at least seven countries (see section 16 for a listing). The prevalence of chronic use and dependence of *N*-pyrrolidino isotonitazene has not been reported.

14. Nature and Magnitude of Public Health Problems Related to Misuse, Abuse, and Dependence

Since its emergence as a novel psychoactive substance in 2024, *N*-desethyl-etonitazene was analytically confirmed in a post-mortem sample collected in Finland in May 2025 (Kriikku et al., 2025). The degree to which the substance was responsible for the death is unclear, as other drugs were detected in the samples from the decedent. *N*-pyrrolidino isotonitazene also produced serious nonfatal harm to individuals in Australia and the Netherlands. (Curtis et al., 2025; Balster et al., 2025). *N*-pyrrolidino isotonitazene was also detected in five toxicology samples analyzed in the US in 2024 and the first half of 2025 (Center for Forensic Science Research and Education, 2025). CFSRE collected these samples from one or more of the following situations: recreational drug use, medicolegal death investigations, clinical intoxications, and/or driving under the influence of drugs investigations.

15. Licit Production, Consumption, and International Trade

No information was found.

16. Illicit Manufacture and Traffic and Related Information

Countries in which the presence of *N*-pyrrolidino isotonitazene has been reported are Australia (Curtis et al., 2025), Finland (Kriikku et al., 2025), Latvia (United Nations Office on Drugs and Crime, 2025), the Netherlands (Balster et al., 2025), New Zealand (High Alert, 2024), the United Kingdom (Vandeputte and Stove, 2025), and the USA (Drug Enforcement

Administration, 2025). Information on the illicit manufacture and traffic of *N*-pyrrolidino isotonitazene specifically is difficult to parse, as most reports present data on nitazenes as a group; however, a report from the National Forensic Laboratory Information System (NFLIS) in the US is an exception: twelve encounters specifically with *N*-pyrrolidino isotonitazene occurred in nine states in the US since 2023 (Drug Enforcement Administration, 2025). The NFLIS database contains cases in which a substance was analyzed as part of an investigation of drug trafficking, distribution, or abuse. *N*-pyrrolidino isotonitazene has been detected in falsified prescription opioid tablets (High Alert, 2024), in substances advertised as another opioid (Curtis et al., 2025), and in mixtures with other non-opioid novel psychoactive substances (e.g., benzodiazepines) (Drug Enforcement Administration, 2025).

17. Current International Controls and Their Impact

N-pyrrolidino isotonitazene has not been formally reviewed by WHO and is not currently under international control.

18. Current and Past National Controls

In 2025, an order for the temporary placement of *N*-pyrrolidino isotonitazene under Schedule 1 of the US Controlled Substances Act was published (Drug Enforcement Administration, 2025). *N*-pyrrolidino isotonitazene is also controlled in Canada and the United Kingdom through regulations controlling nitazenes as a chemical class.

19. Other Medical and Scientific Matters Relevant for a Recommendation on the Scheduling of the Substance

None

References

BALSTER, R., HONDEBRINK, L., BOEL, T. T., VAN DE VELDE, B., VAN DER LINDEN, P. D. & BUTTERHOFF-TERLINGEN, M. H. 2025. [Intoxication with a nitazene; new potent synthetic opioids on a rise]. *Ned Tijdschr Geneeskd,* 169.

CAPRARI, C., FERRI, E., ROSSETTI, P., GREGORI, A., CITTI, C. & CANNAZZA, G. 2025. The emergence of nitazenes: a new chapter in the synthetic opioid crisis. *Arch Toxicol*.

CAYMAN CHEMICAL. 2025. *N-Pyrrolidino Isotonitazene (citrate)* [Online]. Available at: https://www.caymanchem.com/product/34909. [Accessed June 24, 2025].

CENTER FOR FORENSIC SCIENCE RESEARCH AND EDUCATION. 2025. *NPS Opioids in the United States: Trend Reports* [Online]. Available at: https://www.cfsre.org/nps-discovery/trend-reports/nps-opioids. [Accessed July 29, 2025].

CURTIS, B., LAWES, D. J., CALDICOTT, D. & MCLEOD, M. D. 2025. Identification of the Novel Synthetic Opioid N-Pyrrolidino Isotonitazene at an Australian Drug Checking Service. *Drug Test Anal*.

DE VRIEZE, L. M., WALTON, S. E., POTTIE, E., PAPSUN, D., LOGAN, B. K., KROTULSKI, A. J., STOVE, C. P. & VANDEPUTTE, M. M. 2024. In vitro structure-activity relationships and forensic case series of emerging 2-benzylbenzimidazole 'nitazene' opioids. *Arch Toxicol,* 98**,** 2999-3018.

DRUG ENFORCEMENT ADMINISTRATION 2025. Schedules of Controlled Substances: Temporary Placement of Seven Benzimidazole-Opioids in Schedule 1. *Fed Regist,* 90**,** 27268-27273.

GATCH, M. B., HILL, R. D., SUNDAR, M. & SHETTY, R. A. 2025. Discriminative stimulus and antinociceptive effects of sixteen nitazene analogs in rodents. *Neuropharmacology,* 272**,** 110416.

HIGH ALERT. 2024. *Fake oxycodone tablets containing potent synthetic opioid detected* [Online]. Available at: https://www.highalert.org.nz/alerts-and-notifications/fake-oxycodone-tablets-containing-potent-synthetic-opioid-detected/. [Accessed 7 August 2025].

JANOWSKY, A. 2023. N-Pyrrolidino Isotonitazene (citrate): Binding and Functional Activity at Delta, Kappa and Mu Opioid Receptors. Portland, OR: Oregon Health & Science University.

KOZELL, L. B., ESHLEMAN, A. J., WOLFRUM, K. M., SWANSON, T. L., BLOOM, S. H., BENWARE, S., SCHMACHTENBERG, J. L., SCHUTZER, K. A., SCHUTZER, W. E., JANOWSKY, A. & ABBAS, A. I. 2024. Pharmacologic Characterization of Substituted Nitazenes at μ, κ, and Δ Opioid Receptors Suggests High Potential for Toxicity. *J Pharmacol Exp Ther,* 389**,** 219-228.

KRIIKKU, P., PELANDER, A., JYLHÄ, A. & OJANPERÄ, I. 2025. Post-Mortem Identification and Toxicological Findings of Fluetonitazepyne and Isotonitazepyne. *Drug Testing and Analysis*.

REDDIT. 2025. *Isotonitazepyne* [Online]. User reports available at: https://www.reddit.com/r/Opioid_RCs/search/?q=isotonitazepyne&cId=7eea270e-0f6a-49d2-ba00-0f51d1aeedde&iId=0a4c3bc1-469d-4ccc-806f-ff5f2d91d9f7. [Accessed August 9, 2025].

UNITED NATIONS OFFICE ON DRUGS AND CRIME. 2025. *Early Warning Advisory (EWA) on New Psychoactive Substances: Isotonitazepyne* [Online]. Logged into the site at https://www.unodc.org/LSS/Home/NPS and used the search term "isotonitazepyne". [Accessed July 29, 2025].

VANDEPUTTE, M. M. & STOVE, C. P. 2025. Navigating nitazenes: A pharmacological and toxicological overview of new synthetic opioids with a 2-benzylbenzimidazole core. *Neuropharmacology,* 275**,** 110470.

VANDEPUTTE, M. M., VAN UYTFANGHE, K., LAYLE, N. K., ST. GERMAINE, D. M., IULA, D. M. & STOVE, C. P. 2021. Synthesis, Chemical Characterization, and μ-Opioid Receptor Activity Assessment of the Emerging Group of “Nitazene” 2-Benzylbenzimidazole Synthetic Opioids. *ACS Chemical Neuroscience,* 12**,** 1241-1251.

WALTON, S. E., JUHASCIK, M., HARSHBARGER, K., STANG, B. S., REYES, A. G., BAKER, S. M., LOGAN, B. K. & KROTULSKI, A. J. 2024. *N-Pyrrolidino Isotonitazene — NPS Discovery New Drug Monograph,* United States, Center for Forensic Science Research and Education.