
INVESTIGATORS BROCHURE

Investigational Product Company Code: UNI911 (Nasal Spray Solution 1%)

International Non-proprietary Name (INN): Niclosamide Ethanolamine

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26 Aug 2020

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ABBREVIATIONS

ACE	Angiotensin-converting enzyme
AD	Atopic dermatitis
AE	Adverse events
ATx201	Working name for the active drug substance niclosamide
BCOP	Bovine Corneal Opacity and Permeability
BSA	Body Surface Area
COX	Cyclooxygenase
CFU	Colony forming unit
C _{max}	Maximal concentration in plasma
CC	Cytotoxic concentration
COVID-19	Coronavirus disease 2019
EPA	Environmental Protection Agency
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
H. influenzae	Haemophilus influenza
H. pylori	Helicobacter pylori
IC ₅₀	Inhibitory concentration of 50% of effect
IL	Interleukin
LD ₅₀	Lethal Dose causing death of 50% of tested group
LPS	Lipopolysaccharide
M. catarrhalis	Moraxella catarrhalis
MIC	Minimal inhibitory concentration
MIC ₅₀ or ₉₀	Minimal inhibitory concentration inhibiting 50% or 90% of a set of strains
MPE	Mean photo effect
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MSSA	Methicillin-sensitive <i>Staphylococcus aureus</i>
NOAEL	No-observed-adverse-effect level
OTUs	Operational taxonomical units
PEG400	Polyethylene glycol 400
PIF	Photoirritancy factor
RT-PCR	Reverse transcription polymerase chain reaction
PK	Pharmacokinetic
S. aureus	Staphylococcus aureus
SARS-CoV	Severe acute respiratory syndrome coronavirus
SIRS	Skin Infection Rating Scale
S. pneumoniae	Streptococcus pneumoniae
S. pyogenes	Streptococcus pyogenes

STAT3	Signal Transducer And Activator Of Transcription 3
TAA	Treatment Areas Assessment
TLDA	TaqMan Low-Density Array
T _{max}	time to maximum concentration
TPA	tetradecanoyl phorbol acetate
TSS	Total Sign Score
UNI911	Working name for the drug substance niclosamide ethanolamine salt
WHO	World Health Organization

1 SUMMARY

UNION therapeutics A/S, referred to in this document as UNION, is developing UNI911 as intranasal application for prophylaxis in patients at particularly high risk of COVID-19 and its complications.

The active drug substance in UNI911 is the compound niclosamide, as the ethanolamine salt, to increase solubility in the proposed formulation. Niclosamide is a salicylanilide introduced into clinics in the early 1960s as an oral anthelmintic and is currently marketed in several European and developing countries. Orally administered niclosamide is a well-known molecule about which substantial information is publicly available.

In present material, the term “niclosamide” or “niclosamide ethanolamine” will be used to describe public available information related to the compound, whereas information about the compound described by UNION will be referred to as ‘ATx201’ (niclosamide) or ‘UNI911’ (niclosamide ethanolamine salt).

UNI911 Nasal Spray Solution 1% developed by UNION has besides its pleiotropic activities very potent anti-SARS-CoV-2 properties, making UNI911 a promising candidate as a prophylactic treatment for patients at particularly high risk of COVID-19 and its complications. Niclosamide has been identified as the leading candidate for activity against SARS-CoV-2 in two separate library screens of existing approved drugs (Giacca et al; 2020, unpublished data). Furthermore, researchers at Institute Pasteur Korea have reported niclosamide as one of the most potent FDA approved inhibitors of SARS-Cov-2 in in vitro assays using Vero cells, with IC₅₀ of 0.28µM >25x higher than that of chloroquine and >40x higher than that of remdesivir (Jeon et al., 2020).

Thus, in vitro data indicating potent inhibition of SARS-CoV-2 replication and cellular penetration, together with evidence that SARS-CoV-2 initially replicates predominantly in the nasal epithelium, suggests that UNI911 Nasal Spray Solution 1% is best placed as a prophylactic agent or for treatment of early stage COVID-19 disease when the viral load is a main issue.

1.1 Clinical Studies

Even though no clinical trials have yet been conducted in humans for COVID-19, substantial preclinical and clinical data are published supporting the safety of niclosamide.

Niclosamide (tradenames are for instance Yomesan®, Tredemine®) is currently approved and marketed for the oral treatment of tapeworm infections with administration of a single 2 g regimen or 2 g daily for 7 days in adults and children (> 2 years of age). The PK analysis revealed that after oral administration, between 2-25% of the administered dose was detected in the urine, which can be considered as the minimum level of absorption

(Fachinformation Yomesan®). When treating human volunteers each with a single oral dose of 2,000 mg niclosamide, maximal serum concentration of niclosamide was equivalent to 0.25-6.0 µg/mL (Fachinformation Yomesan®). The wide concentration range was caused by the intra-individual absorption differences. Niclosamide is only partially absorbed from intestinal tract, and the absorbed part is rapidly eliminated by the kidneys. Niclosamide has several other weaknesses such as low absorption and oral bioavailability (F = 10%) which may hamper its extensive clinical development as a systemic agent (Chang *et al.*, 2006).

No safety concerns have been detected throughout its >40-year usage: it does neither affect renal nor hepatic function, it is not substantially absorbed by the gastrointestinal tract, mutagenic effects are not expected and occurred adverse events include mainly gastrointestinal tract signs or symptoms (Nausea, gastrointestinal pain, abdominal pain, gagging, diarrhoea). Other adverse events reported with this drug include: immune system disorders (Allergic reaction (e.g. erythema, pruritus and rash), anaphylactic reaction and anaphylactic shock¹), nervous system disorders (Dizziness), angiopathy (Cyanosis), diseases of the, skin and subcutaneous tissue disorders (Skin rash, pruritus, hyperhidrosis) and general disorders and administration site conditions (Malaise (fatigue)). Regarding its use during pregnancy, if possible, treatment with Yomesan® should be started after giving birth.

Moreover, five clinical trials have been completed by UNION that investigated the safety, as well as antimicrobial and anti-inflammatory efficacy of the topical application of niclosamide to skin.

Completed Healthy Volunteer Studies

Study ATx201-001 examined the safety of 3 separate ATx201 2% dermal formulations in 30 healthy volunteers and found no evidence for irritation or other safety signals following 7 days of treatment. A PK analysis showed minimal systemic exposure of ATx201 while local exposure to the skin was substantial.

An irritation study (ATx201-006) in 36 healthy volunteers was conducted with either ATx201 GEL 2% or 4%, on intact and abraded skin, following 21 days with daily application. When applied on abraded skin, approximately half of the subjects in ATx201 2% and 4% and the majority of subjects with ATx201 placebo (84.4%) had mean irritation scores less than 0.5 with these test articles, with only a few (< 10%) subjects higher than 2.0, indicating slight irritation. On abraded skin conditions, ATx201 2% and 4% showed higher cumulative irritation scores than ATx201 placebo; however, the cumulative irritation scores were lower than the negative control and the majority (~ 90%) of subjects

¹ These reactions have been linked to release of dead tapeworm antigens and are hence not idiosyncratic

had scores from 0 to 2.0 on abraded skin suggesting no irritation to slight irritation. No dose-dependent irritation was observed for ATx201 at the tested concentrations. The negative control on abraded skin under occlusive conditions induced the greatest irritation compared to all other test articles in this skin condition. This is expected and serves as an internal control and validation for the study. For these two test articles/skin conditions, the greatest proportion (94.3% of subjects with positive control on intact skin and 77.1% of subjects with negative control on abraded skin) of the subjects had mean irritation scores between 0.5 and 2, indicating slightly irritating products. Concluding, ATx201 GEL showed good tolerability results, with no adverse drug reactions observed across all test articles and skin conditions.

A sensitization study (ATx201-005) in 240 subjects found ATx201 GEL 2% and ATx201 GEL 4% were well tolerated and treatment did not show evidence of sensitization by the investigational products. Overall, no evidence of irritation or sensitization potential was observed with the GEL 2% or 4% formulation. In summary, clinical studies conducted in 306 healthy volunteers support that ATx201 GEL is well tolerated in both 2% and 4% formulations.

Completed Phase 2 Studies

Three Phase 2 studies have been conducted in subjects with either AD or impetigo. The first study (ATx201-002) found that ATx201 GEL 2% applied once- or twice-daily for 7 days was well-tolerated in 36 subjects with AD. Treatment success rates were high with a significant decline in colony counts of *S. aureus* on the skin (AD lesion) in the twice-daily regimen group of subjects with AD, the primary endpoint of the study. A PK analysis showed that systemic concentration 1 hour after the last application was a mean of 1.64 ng/mL in the twice-daily group, while the once-daily group had a systemic level of 0.789 ng/mL. Additionally, application of the ATx201 GEL 2% is associated with increasing the diversity of the skin microbiota on the AD lesions of these subjects, showing a recovery of the commensal flora associated with eradication of *S. aureus*. Another study (ATx201-003) examined the impact of ATx201 Anhydrous CREAM 2% in 31 subjects with AD on inflammation and skin barrier biomarkers. Subjects received once-daily application for 3 weeks. An analysis of lesional skin biopsies for skin barrier and inflammation analysis by immunohistochemistry, reverse transcription polymerase chain reaction (RT-PCR), and microarrays showed that the ATx201 Anhydrous CREAM 2% group had decreases in immune cell infiltrates and down regulation of inflammation biomarkers compared with the vehicle group. Changes in inflammation biomarkers and immune cells correlated with TSS and TAA clinical scores. In the third Phase 2 study in subjects treated for impetigo (ATx201-004, ≤ 5 -day treatment), results show no safety issues; however, no clinical benefit of ATx201 GEL over the vehicle was observed. Furthermore, UNION found no evidence for COX-1 or -2 inhibition in this study supporting the fact that UNI911 has a different anti-inflammatory mechanism from the NSAIDS such as ibuprofen.

Furthermore, UNION has previously conducted a PK Substudy which is part of the currently ongoing Phase 2b study, investigating ATx201 OINTMENT 4% and 7% in patients with atopic dermatitis. In the PK Substudy patients were topically treated (product applied either to 18% or 36% BSA) with ATx201 OINTMENT 7% for two weeks. The maximum concentration (C_{\max}) of ATx201 in subjects with the treated body surface area (BSA) of 18% ranged from 0.5 to 4.4 ng/mL with mean (SD) of 2.0 (1.2) ng/mL on Week 1 Day 1, and ranged from 3.8 to 28.0 ng/mL with mean (SD) 13.1 (8.7) at end of treatment (Week 2 Day 7). For subjects with treated BSA of 36% the C_{\max} of ATx201 ranged from 1.0 to 27.3 ng/mL with mean (SD) of 6.78 (8.5) ng/mL on Week 1 Day 1 and 3.4 to 44.4 ng/mL with mean (SD) of 16.81 (13.1) ng/mL at end of treatment. The C_{\max} after topical administration for 36% BSA on W2D7 (range 3.4 to 44.4 ng/mL) was multiple fold lower than the maximum plasma concentrations observed after oral administration (200 - 6000 ng/ml) as reported in the European SmPC. The median (range) of time to reach maximum concentration (T_{\max}) in subjects with treated body surface area of 18% was 6.1 (2.1 to 8) hrs., on Week 1 Day 1 which is higher than 1.7 (0 to 4.1) hrs, at end of treatment. For the group with treated BSA of 36%, the median (range) of time to reach maximum concentration (T_{\max}) was 8.0 (4.1 to 8.1) hrs., on Week 1 Day 1 which is also higher than 0.5 (0 to 8.0) at end of treatment.

Another clinical study investigated the safety and efficacy of 2g of oral niclosamide patients with mCRC progressing under standard therapy (NIKOLO) (Burock 2018). Interim data of 5 patients showed that after oral intake niclosamide plasma levels peaked after 120 minutes - 720 minutes, with a median C_{\max} plasma level of 0.665 $\mu\text{g/ml}$ (0.429 to 0.848). No drug related toxicities were observed further supporting the promising safety profile of niclosamide.

Niclosamide ethanolamine has not previously been approved as a human pharmaceutical drug. By using niclosamide ethanolamine the solubility is increasing from 6.5 mg/L to 230 mg/L (Gönnert 1961), thus increasing the exposure to the target tissue affected by COVID-19. Although niclosamide ethanolamine is not an approved salt form in humans, the ethanolamine salt of niclosamide is used in formulations approved for use as a molluscicide by the US-Environmental Protection Agency (EPA 1999) under various trade names (e.g., Bay 73, Bayuscide) (Andrews *et al.*, 1983). The niclosamide ethanolamine used in these approved formulations has the common name, clonitralid and inhaled toxicology studies have been conducted with niclosamide ethanolamine.

Ethanolamine is listed as an available salt for use in pharmaceuticals (Gupta *et al.*, 2018). Ethanolamine has been used as an approved excipient in sterile dosage formulation at levels up to 0.15% (Niazi 2016) and has been reported to be used as a salt for ascorbic acid in intramuscular formulations (Knaak *et al.*, 1997). Ethanolamine has been studied as a counter ion for non-steroidal anti-inflammatory compounds (Cheong *et al.*, 2002).

1.2 Nonclinical Studies

The nonclinical pharmacology and/or safety of niclosamide (free acid), niclosamide ethanolamine, and ethanolamine alone have been evaluated in several studies published over the past 5 decades, including single-dose and repeat-dose oral administration studies. UNION has conducted various nonclinical studies with ATx201, including several microbiological in vitro activity studies, and 2 Good Laboratory Practice (GLP) studies on the phototoxic potential (1 in mice and 1 in vitro). Additionally, two bridging pharmacokinetic/toxicokinetic studies (1 in rats and 1 in dogs) with ATx201 and UNI911 were recently conducted by UNION.

Niclosamide has been shown to inhibit SARS-CoV-2 infection in Vero Cells with an IC₅₀ of 280 nM in the absence of cytotoxicity (> 50 µM) (Jeon 2020). Interestingly, in this screening niclosamide was identified as the most potent compound in their collection with a potency >30x higher than Gilead's compound Remdesivir and >20x more potent than the anti-malaria drug Chloroquine which are both being currently tested in clinical trials.

UNION has demonstrated anti-bacterial potency against a variety of bacteria important in respiratory disease, incl. multi-drug resistant strains of *S. aureus* (incl. MRSA), *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, and *S. pyogenes*. Extensive data is shown in [Section 4.1.1.4](#). Furthermore, the novel antibacterial mode of action of niclosamide favours the low likelihood of evolving resistance, which has been shown for *S. aureus*.

Moreover, an *in vivo* study in mice with niclosamide demonstrated reduction of mucus production and secretion as well as bronchoconstriction and showed additional anti-inflammatory effects (Cabrita *et al.*, 2019).

Finally, published literature indicates no identifiable hazards from niclosamide in repeat-dose general toxicity, reproductive toxicity, and genotoxicity/carcinogenicity studies. Since these older toxicity studies did not measure plasma levels of niclosamide or its reduced metabolite (2,5'-dichloro-4'-amino-salicylanilide), UNION conducted 2 bridging pharmacokinetic/toxicokinetic studies at dose levels in the range administered to rats and dogs during these previous toxicity studies. The rat bridging study found large plasma margins for niclosamide (up to 23-fold relative to human Yomesan exposure) at NOAELs reported in the previous toxicity studies, indicating the oral gavage route to be robust for toxicity evaluations in rats.

Genotoxicity and carcinogenicity of niclosamide have been well described in the literature including a National Cancer Institute (NCI) report. The overall conclusion on genotoxicity by UNION is the same conclusion drawn by the Environmental Protection Agency (EPA 1999), which concluded that niclosamide showed no evidence of causing genotoxicity. Most important is that 2 carcinogenicity studies conducted with niclosamide ethanolamine

have been published by the NCI and showed no evidence of carcinogenicity in mice and rats. UNION also conducted a GLP in vivo skin epidermal micronucleus evaluation in minipigs with ATx201 using validated methodology and showed no effects on micronucleus formation after twice daily applications of a 4% formulation for 28 days.

Several studies have assessed the reproductive toxicity of niclosamide and reported that niclosamide does not show significant reproductive toxicity. No embryotoxic or teratogenic effects were seen in either rats or rabbits after administration of 1000 mg/kg/day orally during periods of organogenesis. No other reproductive toxicity studies have been conducted by UNION.

UNION also assessed ATx201 formulations up to 4% strength for ocular irritation and phototoxicity potential. These tests identified no potential of ATx201 formulations to cause ocular irritation or phototoxicity.

2 INTRODUCTION

UNI911 Nasal Spray Solution 1% represents a potent prophylactic agent against SARS-CoV-2 for subjects at significant risk, delivered directly to the nasal cavity. UNI911 is an formulation for intranasal application with the active ingredient niclosamide (5-chloro-N-[2-chloro-4-nitrophenyl]-2-hydroxybenzamide), a salicylanilide introduced in the early 1960s as antiparasitic tablets against parasitic diseases in humans including pregnant women and children (Pearson *et al.*, 1985, Ofori-Adjei *et al.*, 2008) and veterinary medicine. Importantly, the compound has only been approved by FDA as oral anti-parasitic treatment, not yet as an inhalable treatment. The drug is listed in the WHO Model List of Essential Medicines 2015 and the WHO Model Formulary 2008.

Niclocide® (niclosamide), an oral anthelmintic, was approved in the US (Niclocide; NDA 018669, Bayer Pharmaceuticals; May 14, 1982) for the treatment of tapeworm infections with administration of a single 2 g regimen or 2 g daily for 7 days. Niclocide marketing was discontinued in 1996 for commercial reasons; the NDA holder notified the FDA that the drug product was no longer marketed and requested that the approval of the application be withdrawn (Federal Register Vol. 61, No.192 pg. 51457). Oral niclosamide is still currently marketed in developing countries including South Africa, and in several European countries, including Finland, France, Germany, Netherlands, and Sweden under the trade names Yomesan, Kontal, and Tredemine for treatment of children and adults.

The safety profile of niclosamide is well established due to decades of safe oral use taken by patients – incl. children and pregnant women – in doses of 2g / day. Also, inhalation studies in rodents have demonstrated LD50 of >2000 mg / m³ / 1 hour. Furthermore, UNION has demonstrated topical safety in a range of preclinical and clinical models on the skin and in the eye with consistently supportive results.

Besides its activity against SARS-CoV-2, the antiviral spectrum of niclosamide includes a variety of RNA and DNA viruses, such as ebola, zika, chikungunya, enterovirus, influenza, human rhinoviruses and adenovirus (Saiz *et al.*, 2017, Wang *et al.*, 2016, Jurgait *et al.*, 2012, Xu *et al.*, 2016, Marrugal-lorenzo *et al.*, 2019, Madrid *et al.*, 2015). Notably, it was found that niclosamide displays promising inhibitory activity against SARS-CoV replication, a closely related virus to SARS-CoV-2, with an EC₅₀ value of less than 0.1 µM in Vero E6 cells and against MERS-CoV replication by up to 1000-fold at 48 h p.i. at a concentration of 10 µM in Vero B4 cells (Xu *et al.*, 2019).

UNION has also demonstrated antibacterial potency of niclosamide against a variety of bacteria incl. the most prevalent bacteria causing pneumonia, such as multi-drug resistant strains of *S. aureus* (incl. MRSA), *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, and *S. pyogenes* (see [Section 4.1.1.4](#)).

Furthermore, UNION has identified broad non-steroidal anti-inflammatory effect in preclinical and clinical models. In particular, a post-hoc analysis of the clinical study ATx201-003 revealed that the breadth of biomarkers modulated by ATx201 is on par with a moderate steroid (betamethasone). Furthermore, research groups have demonstrated the anti-inflammatory benefit of niclosamide in models including an asthmatic mice model that showed reduction of mucus production and secretion as a result of treatment with niclosamide (Cabrita *et al.*, 2019).

Niclosamide is currently under investigation in several academic centers in a wide variety of other conditions, ranging from different cancers, inflammatory diseases, and viral infections.

UNION is also currently developing niclosamide as a topical treatment of atopic dermatitis (ATx201 OINTMENT in Phase 2b) and oral treatment for *H. pylori* treatment.

2.1 Indication Under Investigation

COVID-19 is a viral infection caused by a newly discovered coronavirus SARS-CoV-2, a positive single-stranded (RNA) beta coronavirus. SARS-CoV-2 represents the causative agent for a potentially fatal disease of huge global health concern. Due to the recent discovery of this specific virus, there is limited knowledge about the epidemiology as well as the long-term consequences such as sequela. To date, there are 3,526,178 confirmed cases and 247,971 deaths reported worldwide, and these numbers are still rising (04.05.2020, John Hopkins University).

Most people infected with SARS-CoV2 will experience mild to moderate respiratory illness affecting the airways by induction of inflammation and in some cases bacterial super-infection. In some cases, COVID-19 might cause severe pneumonia and in the most

critical cases COVID-19 might lead to acute respiratory distress syndrome (ARDS). COVID-19 leads to hospitalization at intensive care units in 12% of cases and death in approximately 2.3% of the cases (Novel 2020, Grasselli *et al.*, 2020).

Occurrence of secondary infections or bacterial super-infections in COVID-19 patients has been described to be of concern, however the exact rate remains unknown (Zhou 2020). For instance, a small study from a hospital in Wuhan and Jinyintan found that 50% of COVID-19 deaths had secondary infections (Zhou *et al.*, 2020). Furthermore, it is thought that bacterial superinfection with *S. aureus* and *S. pneumoniae* account for an increase in morbidity and mortality and seems to increase after three weeks on the ventilator. Finally, secondary superinfection has been described as one of the predictors of a fatal outcome of COVID-19 (Ruan *et al.*, 2020).

The pathogenesis of COVID-19 seems to be complex and mostly unknown. However, it is thought that SARS-CoV2 accesses the type II alveolar epithelial cells of the lungs via ACE2 causing an excessive immune reaction in the host leading to severe pneumonia and respiratory distress (Tai *et al.*, 2020, Rothan *et al.*, 2020). Additional findings are leucopenia and significantly high blood levels of proinflammatory cytokines and chemokines, such as IL1 β , IL8, IL10, IFN γ , CCL3 and TNFa (Rothan *et al.*, 2020).

At present, no specific anti-viral drugs or vaccines exist against COVID-19 infection as potential therapy of humans. The only approved option is using the HIV-protease inhibitor and Nucleoside inhibitors to attenuate the viral infection and it is clear that more research is urgently needed to identify novel therapeutic drugs to treat COVID-19. The symptomatic treatment at hospitals is currently respiratory support e.g. ventilator, observation and antibiotics to minimize the risk of a bacterial super-infection. Moreover, inhalable steroids are frequently used to reduce the risk of inflammation caused by the viral infection and that might destroy the lung tissue leading to hypoxemia.

2.2 UNI911 Inhalation and Nasal Application

UNI911 represents a novel triple mechanism of action including antiviral, antibacterial and anti-inflammatory properties. In particular, UNION finds the activity of niclosamide of significant interest as an ‘all-in-one’ solution to 1) eliminate COVID-19 in the airways, 2) decrease respiratory inflammation and 3) reduce risk of bacterial super-infections leading to fatale pneumonia:

- Niclosamide has demonstrated efficacy *in vitro* against SARS-CoV-2 with an IC₅₀ of 280 nM, which is 30-fold below the IC₅₀ of the promising COVID-19 treatment from Gilead in Phase 3 (Jeon *et al.*, 2020). A more detailed description of this data can be found in [Section 4.1.1.1](#).

- Niclosamide was also reported to present anti-inflammatory properties *in vitro* by modulating the activation of dendritic cells and repressing the expression of proinflammatory cytokines (Wu *et al.*, 2014). Moreover, niclosamide was found to be a potent inhibitor of STAT 3, a signaling pathway involved in production of variety of cytokines (Ren 2010). Furthermore, niclosamide's anti-inflammatory activity have been validated in a murine model of asthma alongside with demonstrating reduction of mucus secretion and bronchoconstriction upon treatment with niclosamide (Cabrita *et al.*, 2019, see [Section 4.1.1.3](#)). Finally, it was shown that topically applied ATx201 significantly decreases the expression of a breadth of inflammatory biomarkers across several T cell lineages in patients with moderate atopic dermatitis (ATx201-003 Study).
- UNION conducted studies that have demonstrated anti-bacterial potency of ATx201 against a variety of airway related bacteria incl. multi-drug resistant strains of *S. aureus* (incl. MRSA), *S. pneumoniae*, *H. influenzae*, *S. pyogenes* and *M. catarrhalis*, exerting a stable MIC distribution in such (see [Section 4.1.1.4](#)). Especially regarding the efficacy against *S. aureus*, a clinical trial conducted by UNION showed that topically applied niclosamide (ATx201) significantly reduces *S. aureus* colonization and increases skin microbiome diversity in patients with mild-to-severe atopic dermatitis (DECOLAD II).

The novel triple mechanism of action is expected to attenuate the activity of SARS-CoV-2 in the airways, reduce pathogenic bacteria that worsen the respiratory insufficiency, reduce the inflammatory processes affecting the respiratory tissue leading to decreased oxygenation of the blood. This, together with the promising safety profile indicates that UNI911 could become a potential treatment candidate to cure the respiratory collapse observed in a high number of patients suffering from COVID-19. UNI911 would function through three mechanisms that seem to be pivotal in the development of respiratory insufficiency caused by SARS-CoV-2. Furthermore, *in vitro* data indicating potent inhibition of SARS-CoV2 replication and cellular penetration, together with evidence that SARS-CoV-2 initially replicates predominantly in the nasal epithelium, suggests that UNI911 is best placed as a prophylactic agent or for treatment of early stage COVID-19 disease when the viral load is a main issue.

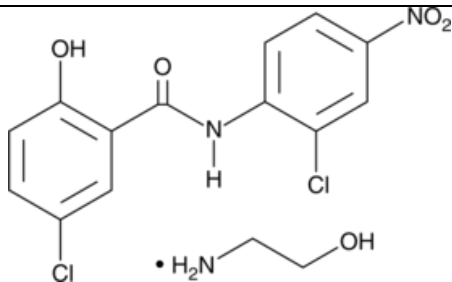
3 PHYSICAL, CHEMICAL AND PHARMACEUTICAL PROPERTIES AND FORMULATION

3.1 Pharmaceutical Presentation

The active drug substance of UNI911 (niclosamide) is adopted in the European Pharmacopeia with 2 monographs; 1 for niclosamide anhydrous and 1 for niclosamide

monohydrate. UNION inhalable formulation of UNI911 contains niclosamide ethanolamine salt.

3.2 Physical and Chemical Properties of the Drug Substance

UNION Compound Number:	UNI911
Approved Name (USAN and INN):	Niclosamide ethanolamine salt
Chemical Name (IUPAC):	5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxy-benzamide compd. with 2-aminoethanol
Molecular Formula:	C ₁₃ H ₈ Cl ₂ N ₂ O ₄ .C ₂ H ₇ NO
Molecular Weight:	388.2 g/mol
Physical Form:	Yellow solid
Structural formula	

3.3 Formulations, Including Excipients

The investigational medicinal product (IMP) will be manufactured according to Good Manufacturing Practices (GMP)

The composition of UNI911 Nasal Spray Solution 1% is detailed in [Table 1](#). All excipients used are of compendial quality (*Ph. Eur.*) and none are of human or animal origin.

Table 1: Composition of UNI911 Nasal Spray Solution 1%

Ingredient	Quantity (g)	Function	Reference monograph
Niclosamide ethanolamine	1.00 ¹⁾	Drug substance	In-house
PVP (Kollidon K30)	2.00	Stabilizing agent	Ph. Eur.
Cyclodextrin (HP-β-CD) (Kleptose HPB)	15.00	Solubility enhancer	Ph. Eur.
NaOH	0.2	pH adjustment	Ph. Eur.
1N HCl	ca. 4.0	pH adjustment	Ph. Eur.
Water for Injection	QS up to	Solvent	Ph. Eur.
Total	100.00		

UNI911 Nasal Spray Solution 1 % contains 10 mg/g niclosamide ethanolamine equivalent to 8,4 mg/g of niclosamide free base

4 NONCLINICAL STUDIES

4.1 Non-clinical Pharmacology

4.1.1 Primary Pharmacology

4.1.1.1 *In Vitro* Antiviral Efficacy of niclosamide

Niclosamide has been shown to exhibit antiviral activity against SARS-CoV-2 in Vero cells with an IC_{50} of 0.28 μ M and $CC_{50} > 50 \mu$ M, resulting in a Selectivity Index of 176.65 (Vero cells were infected with SARS-CoV-2 at a multiplicity of infection (MOI) of 0.0125 and the inhibition of niclosamide was measured using immunofluorescence normalized to the negative control DMSO; [Figure 1](#)) (Jeon *et al.*, 2020). The mechanism of action remains to be elucidated; however it was recently demonstrated that niclosamide inhibits SKP2 activity enhancing autophagy and reducing MERS-CoV replication (Xu *et al.*, 2020), hence a similar mechanism might be attributable to the activity of niclosamide on SARS-CoV-2.

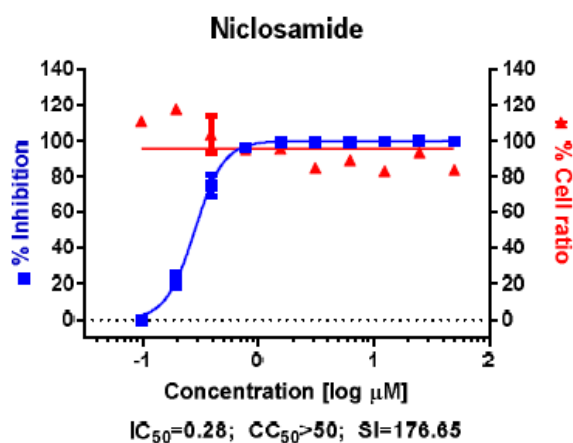


Figure 1: Dose-response of SARS-CoV-2 inhibition by niclosamide *in vitro*. The blue squares represent inhibition of virus infection (%) and the red triangles represent cell viability (%).

Besides its activity against SARS-CoV-2, the antiviral spectrum of niclosamide includes other pathogenic beta-coronaviruses, such as SARS-CoV and MERS-CoV. Moreover, niclosamide has shown potent *in vitro* activity at nanomolar to micromolar concentrations against a variety of other viruses, including single-strand RNA viruses, such as human rhinovirus, influenza, zika, ebola, and chikungunya virus (Kadri *et al.*, 2018, Jurgait *et al.*, 2012). Recently, it has been revealed that niclosamide can also block the nuclear translocation of adenovirus (double-stranded DNA) and inhibits DNA replication of cytomegalovirus (Marrugal-lorenzo *et al.*, 2019). The breadth of antiviral effect is summarized in [Table 2](#).

Table 2: Summary of published antiviral effect of Niclosamide

Virus	Number of Serotypes	EC50 Range [μM]	References
Adenovirus	2	0.6-2.3	Marrugal-Lorenzo <i>et al.</i> , 2019
CHIKV	2	0.36-0.95	Wang <i>et al.</i> , 2016
DENV-1	1	1.45-1.5	Jung <i>et al.</i> , 2019; Mazzon <i>et al.</i> , 2019
DENV-2	1	0.38-0.4	
DENV-3	1	0.37-1.6	
DENV-4	1	0.25	Jung <i>et al.</i> , 2019
Ebola Virus	1	1.5	Madrid <i>et al.</i> , 2015
gamma-herpesvirus	1	0.17	Huang <i>et al.</i> , 2017
Hepatitis C	1	0.16 -2	Stachulski <i>et al.</i> , 2011
HSV-1	1	14.6	Jurgeit <i>et al.</i> , 2012
HSV-2	1	0.43	Jurgeit <i>et al.</i> , 2012
Influenza virus	1	0.83	Jurgeit <i>et al.</i> , 2012
Japanese encephalitis virus	1	5.8	Fang <i>et al.</i> , 2013
Kaposi sarcoma associated herpesvirus	1	0.15	Huang <i>et al.</i> , 2017
Rhinovirus	4	0.84-1.38	Jurgeit <i>et al.</i> , 2012
Ross River Virus	1	0.82	Mazzon <i>et al.</i> , 2019
SARS-Coronavirus	1	0.1-1.56	Xu <i>et al.</i> , 2020, Wu <i>et al.</i> , 2004
SFV	1	1.79-3	Mazzon <i>et al.</i> , 2019 Wang <i>et al.</i> , 2016
Sindbis Virus	1	2.8	Mazzon <i>et al.</i> , 2019
SINV	1	1.07	Wang <i>et al.</i> , 2016
Yellow Fever 17D	1	< 0.03	Mazzon <i>et al.</i> , 2019
Zika Virus	1	0.28	Xu <i>et al.</i> , 2016

4.1.2 Secondary Pharmacology

Niclosamide is also under investigation in several academic centers in a wide variety of other conditions, spanning from cancer, autoimmune and metabolic disorders.

First, its antineoplastic efficacy is tested in preclinical and clinical studies, such as head and neck cancer (Li *et al.*, 2014), colorectal cancer (Burock *et al.*, 2018), prostate cancer (Fenner 2014) and ovarian cancer (Li *et al.*, 2014). Secondly, the properties of niclosamide as a potent anti-inflammatory agent were examined for endometriosis (Sekulovski *et al.*, 2019), rheumatoid arthritis (Al-Gareeb *et al.*, 2018), inflammatory bowel disease (Glick *et al.*, 2017), Sclerodermatous Graft-Versus-Host disease (Morin *et al.*, 2016a), systemic sclerosis (Morin 2016a) and renal ischemia/reperfusion injury (Zhang *et al.*, 2017). Thirdly, its action as mitochondrial uncoupler has been exploited to investigate its potency in the context of metabolic disease such as NASH, obesity and diabetes (Al-Gareeb *et al.*, 2017, Guo *et al.*, 2019, Park *et al.*, 2019).

UNION is also currently developing ATx201 in an ointment formulation for the treatment of atopic dermatitis, and is currently in clinic with a Phase 2b study.

Niclosamide is an effective anthelmintic agent and pesticide (Pearson *et al.*, 1985, Ofori *et al.*, Adjei 2008).

4.1.2.1 *In Vitro* Immunological Effects of niclosamide

Niclosamide is a potent inhibitor of STAT 3, a signaling pathway involved cytokine production of majorly Th17 cells and proliferation of immune cells (Ren 2010). Furthermore, it was found that niclosamide inhibits the production of proinflammatory cytokines secreted by several T helper cell lineages, such as Th1, Th2 and Th17, which is important in the light of limiting the excessive production of proinflammatory cytokines in the lung present in the severe state of COVID-19 (Shi *et al.*, 2020). Moreover, Wu *et al.* (2014) found that niclosamide inhibits LPS-induced dendritic cells maturation and cytokine, costimulatory molecule, and MHC molecule expression. Niclosamide-treated dendritic cells further inhibited antigen specific T-cell response. This would further contribute to decrease the elevated cytokine levels observed in COVID-19 (Rothan *et al.*, 2020).

However, its anti-inflammatory properties seem to go beyond STAT 3 inhibition, as early studies link niclosamide's activity to mitochondrial uncoupling and its ability to target multiple signaling pathways, which are involved in a variety of diseases (Weinbach *et al.*, 1969, Frayha *et al.*, 1997); Niclosamide's broad anti-inflammatory activity was shown to be potent as a) the phosphorylation of STAT3/AKT/ β -catenin phosphorylation was suppressed in a mouse skin systemic sclerosis model resulting in reduction of T-helper- and B-cells, b) T-cell activity was inhibited in PBMCs from inflammatory bowel disease patients *ex vivo*, c) proinflammatory cytokine production, such as IL17A and IL6, was decreased in niclosamide-treated human synovial fibroblasts, which was accompanied by suppression of ERK/JNK phosphorylation and d) inflammatory factors in the endometriotic microenvironment were inhibited by targeting STAT3 and/or NF- κ B signaling (Morin *et al.*, 2016b, Shivaji *et al.*, 2019, Liang *et al.*, 2015, Sekulovski *et al.*, 2018). Besides, niclosamide exhibits strong antineoplastic activity by 1) inhibition of STAT3 phosphorylation in colon cancer and head and neck cancer cells, 2) suppression of Wnt/ β -catenin signaling by targeting the Wnt co-receptor LRP6 on the cell surface, an activity that is closely associated with antiproliferation and proapoptotic activities 3) inhibition of the NF- κ B pathway and increase reactive oxygen species levels in acute myelogenous leukemia stem cells; 4) targeting FXR1 and IGF2BP2 in ovarian cancer cells, 5) inhibition of growth of several types of cancer cells with constitutive STAT3 activation (eg, DU145, HeLa, A549); and suppression of mTORC1 signaling through the modulation of cytoplasmic pH in MCF-7 breast cancer cells (Li *et al.*, 2014, Shi *et al.*, 2017, MacLean *et al.*, 2018, Wu *et al.*, 2014).

Thus, the breadth of niclosamide's anti-inflammatory action which spans across multiple immune cell lineages and types is thought to hinder the "cytokine storm" caused by SARS-CoV-2 infection in lung cells occurring in the more severe state of the disease (Shi *et al.*, 2020) and therefore limiting the progression of the disease.

4.1.2.2 *In Vivo* Anti-Inflammatory Effect of niclosamide in asthmatic mice and TPA induced inflammation

In a murine asthma model, induced by Ovalbumin/carbachol (OVA/CCH), intratracheal treatment with niclosamide for 3 days resulted in a significant reduction of intracellular mucus and airway contraction, which has been investigated in 17–40 airway sections (Cabrita *et al.*, 2019). Furthermore, peribronchial immune cell infiltration (CD45+ cells) observed in OVA-induced airway inflammation was strongly reduced by niclosamide. Moreover, Cabrita *et al.* speculated that niclosamide inhibition of TMEM16A or TMEM16F contributes to these effects.

Furthermore, in a tetradecanoyl phorbol acetate (TPA) induced ear inflammation test in mice of ATx201; ATx201 OINTMENT 4% (mean [SD] = 13.78 mg) statistically significantly reduced ear weight compared with placebo (17.49 mg; $p = 0.0266$; Study BA2018-007N). ATx201 CREAM 2% (mean = 0.29 mm; $p = 0.0196$), OINTMENT 2% (0.24 mm; $p = 0.0002$), and OINTMENT 4% (0.24 mm; $p = 0.0002$) statistically significantly reduced ear thickness compared with the placebos (0.39 mm and 0.37 mm, respectively). Ear weight and ear thickness results for the ATx201 formulations were similar to those for Locoid a mild-moderate potency steroid. Further investigations are warranted to establish a clear dose-response relationship.

4.1.2.3 *In Vitro* Antibacterial Effect of niclosamide

UNION has demonstrated that ATx201 has potent anti-bacterial efficacy against a variety of bacteria, including the pathogens that are thought to be most relevant to COVID-19:

- multi-drug resistant strains of *S. aureus* (incl. MRSA) with a MIC distribution of 0.06 – 0.5 µg/mL in 226 isolates (incl. clinical isolates) tested (Study reports ATx0030014, AntibioTx 1 and 3 data, ATx001125, ATx003000)
- *S. pneumoniae* with a MIC of 0.25 µg/mL (Study Report AntibioTx Data 1 and 2)
- *H. influenzae* with a MIC of 0.5 µg/mL (Study Report AntibioTx Data 1)
- *M. catarrhalis* with a MIC of 0.12 µg/mL (Study Report AntibioTx Data 1)
- *S. pyogenes* with a MIC of 0.125 µg/mL (Study Report AntibioTx 2 and 3, ATx0030014)

4.1.2.4 *In Vivo* Antibacterial effect of niclosamide

Several *in vivo* studies were conducted to assess the efficacy of ATx201 dermal formulations against methicillin-resistant and fusidic acid-resistant *S. aureus* using the thoroughly validated murine superficial skin infection model (Vingsbo Lundberg *et al.*, 2013). In this animal model, a MRSA of the USA300 clonal type was used (Study ATx001137).

Over the course of 3 separate animal studies (N=152) it was shown that a wide range of dermal formulations of niclosamide demonstrated a significant reduction in the number of CFUs. Results indicate that ATx201, formulated as a gel, cream, or anhydrous cream is able to produce a statistically significant reduction in MRSA CFUs comparable with that seen with currently approved topical antibiotics (fusidic acid).

A subsequent study was performed (N=64) using a clinical isolate of *S. aureus* resistant to both fusidic acid and methicillin in the murine superficial skin infection model (Vingsbo Lundberg 2013). In this study, different ATx201 OINTMENT formulations containing between 0.5–4% of ATx201 were tested along with the commercially available fusidic acid formulation (Study ATx001331). Results showed that unlike fusidic acid, the OINTMENT 2% and 4% formulations of ATx201 significantly reduced the number of *S. aureus* CFUs. These experiments support the potential benefits of using ATx201 as a topical antibiotic to treat skin infections caused by fusidic acid-resistant and methicillin -resistant strains of *S. aureus*. A subsequent study confirmed the efficacy of ATx201 CREAM (Study ATx002105).

4.2 Pharmacokinetics and Product Metabolism in Animals

The nonclinical absorption, distribution, metabolism, and elimination characteristics of ATx201/niclosamide were established in a variety of experimental models conducted by UNION or described in relevant published studies with niclosamide. The major metabolites of niclosamide, identified in the Wistar rat after oral administration, are the O-glucuronide of niclosamide in bile (hydrolysed by β glucuronidase in the intestines), the 4'-nitro-reduced metabolite, (2',5-dichloro-4'-amino-salicylanilide) in the urine, and unchanged niclosamide in the feces (Griffiths 1979).

4.2.1 Absorption

4.2.1.1 Single-Dose Pharmacokinetics

4.2.1.1.1 Systemic Absorption after Single Oral Administration

In published studies (Duhm, *et al.*, 1961, Andrews *et al.*, 1983), pharmacokinetics were studied in male rats administered a single oral dose of 50 mg/kg ¹⁴C-niclosamide

ethanolamine salt. About one third of the oral dose was estimated to be absorbed in the stomach and intestines and two-thirds was excreted in the feces. The plasma half-life of radiolabel was estimated at 6 hours. Similar plasma half-life was observed for niclosamide following oral administration of 5 mg/kg niclosamide which showed a bioavailability of 10% (Chang *et al.*, 2006).

UNION conducted single-dose bridging pharmacokinetic/toxicokinetic studies in rats and dogs at dose levels spanning those used in several toxicology studies reported by Bayer (Study 19UNTXP2, 2019; Study 19UNTXP1, 2019).

These bridging studies had two purposes:

- To determine systemic exposure of niclosamide and its major metabolite at dose levels investigated in previous toxicology studies.
- To compare systemic exposure of niclosamide when given as the free acid and the ethanolamine salt of niclosamide

In both species, the parent niclosamide and its major metabolite 2,5'-dichloro-4'-amino-salicylanilide, which is formed by reduction of the 4'-nitro moiety of the parent molecule, were monitored over a 96-hour period. Rats were dosed orally via gavage with either niclosamide (free acid) or niclosamide ethanolamine, while dogs received niclosamide free acid orally via capsules.

In both rats and dogs, niclosamide was rapidly absorbed, with systemic exposures (mean AUC values) for niclosamide ranging from 2070 to 29,217 ng·h/mL at dose levels of 50 to 1000 mg/kg in rats, and 63.6 to 950 ng·h/mL at 30 to 1000 mg/kg in dogs. [Table 3](#) summarizes plasma levels of the parent niclosamide for rats and dogs, as well as humans, following a single oral dose, showing that at low, mid and high doses of niclosamide free acid, the AUC in rats is about 2-, 7- and 23-fold higher than the AUC at human Yomesan oral dose of 1 g bid. For dogs the high dose yielded an AUC ~1.5-fold than the AUC at human oral dose of 1 g bid.

Table 3: Pharmacokinetics of Niclosamide in Rats and Dogs after Single Dose Oral Administration of Niclosamide (free acid) or Niclosamide Ethanolamine (Comparison to Human Oral Exposures)

Species	Route	Niclosamide dose form	Dose	AUC (ng·h/mL)	C _{max} (ng/mL)
Rat	Oral ^a	Free acid	50 mg/kg	2070	276
			250 mg/kg	8439	847
			1000 mg/kg	29,217	2203
		Ethanolamine salt	50 mg/kg	5798	1557
			250 mg/kg	11,742	3793
			1000 mg/kg	22,803	6041
Dog	Oral ^b	Free acid	30 mg/kg	63.7	8.93
			100 mg/kg	101	31.0
			300 mg/kg	286	26.8
			1000 mg/kg	950	131
Human	Oral	Free acid	1 gram ^c	631	166
			2 grams ^d	1262 ^d	665

Abbreviations: AUC = area under the plasma concentration-time curve (zero to last measured time point);

C_{max} = maximum plasma concentration

^a From Study 19UNTXP2 after oral gavage in suspension with 0.5% methylcellulose / 0.2% Tween 80.

^b From Study 19UNTXP1 after oral administration via gelatin capsules.

^c From Schweizer *et al.*, 2018.

^d From Burock 2018; AUC following 2 grams estimated from 1 gram dose (Schweizer *et al.*, 2018) assuming dose proportional AUC.

4.2.2 Distribution

Niclosamide is known to interact with plasma proteins with high affinity and high capacity (Maltas 2014); however, the percent of niclosamide bound to plasma protein has not been reported though most all halogenated salicylanilides have extensive (>97%) plasma protein binding (Swan 1999).

After once daily administration of ¹⁴C-labeled niclosamide ethanolamine salt for 7 consecutive days to male rats, no accumulation of niclosamide was found in major tissues/organs evaluated, i.e. gastrointestinal tract, blood, liver, kidney, testes, and adrenals (Duhm *et al.*, 1961, Andrews *et al.*, 1983). The highest levels of ¹⁴C-equivalents were found in organs of excretion (gastrointestinal tract, liver, and kidney), with liver levels slightly greater than blood throughout the approximately 24-hour evaluation period. In addition, a niclosamide solution administered to rats at 2 mg/kg demonstrated peak

niclosamide levels in liver, heart and spleen at the first collection timepoint (2 hours), with niclosamide rapidly cleared from these organs over a 6-hour period (Ye *et al.*, 2014).

4.2.3 Metabolism

4.2.3.1 Niclosamide Metabolism *In Vitro*

Hydroxylation and glucuronidation of niclosamide has been observed using human liver microsomes and individually expressed human cytochrome P450 enzymes (CYPs) and UDP-glucuronosyltransferases (UGTs). The majority of the hydroxylation and glucuronidation activities were mediated by CYP1A2 and UGT1A1 (Lu 2015). The reaction kinetics for hydroxylation and glucuronidation in human microsomes were compared across species using microsomes from mouse, rat, dog, monkey, and minipig. For both enzymatic activities, the intrinsic clearance values for hydroxylation were considerably higher for human microsomes versus all other species. Glucuronidation, but not hydroxylation, appears to be a major *in vivo* elimination pathway in rats and humans (see [Section 4.2.3.2](#)).

Fan *et al.* (2019) demonstrated that niclosamide was much less stable in mouse intestinal versus liver microsomes during incubations optimized to glucuronidate substrates (i.e., contain uridine diphosphate glucuronic acid; UDPGA), indicating intestinal glucuronidation to be important in metabolism of niclosamide in mice.

4.2.3.2 Niclosamide Metabolism *In Vivo*

Results from studies in rodents and humans (Section 5.3) show that the drug is excreted both in the free form and as conjugated glucuronides. In rats and dogs after oral administration, a major circulating plasma metabolite is 2,5'dichloro-4'-amino-salicylanilide, which is formed by reduction of the 4'-nitro moiety of the parent molecule (Study 19UNTXP2, Study 19UNTXP1 2019, Andrews 1983, Duhm 1961, Griffiths 1979). This 4'-nitro reduced metabolite is likely generated via intestinal microflora as experiments in rats demonstrated that the reduced metabolite is: 1) formed during incubations with intestinal microflora under anaerobic conditions, 2) not generated after intraperitoneal administration of niclosamide (absorption by-passes intestines), and 3) attenuated following oral administration of niclosamide to antibiotic-treated rats (Griffiths 1979). The plasma exposure (AUC) of the 4'-nitro-reduced metabolite formed after oral administration to rats and dogs ranges from 63.4 to 2193 ng·h/mL (Study 19UNTXP2; Study 19UNTXP1). Although both rats and dogs demonstrated substantial plasma levels of 2,5'dichloro-4'-amino-salicylanilide, because dogs have less exposure to the parent versus rats, the percent of the 4'-nitro-reduced metabolite relative to the parent is much greater for dogs versus rats.

The metabolism of niclosamide was also studied in pregnant rats orally administered 1000 mg/kg on Day 13, 19, or 20 of pregnancy. Rats were sacrificed 4, 8, 16, or 24 hours after niclosamide administration. Maximal concentrations of niclosamide and the main metabolite 2',5'-dichloro-4'-aminosalicylanilide were found in the liver and kidneys 8 hours after administration. Niclosamide was detected in fetuses from dams treated on Day 13 of pregnancy. No 2',5'-dichloro-4'-aminosalicylanilide was detected. Fetuses from dams treated on Days 19 or 20 of pregnancy contained both niclosamide and the main metabolite. These studies indicate that up to 13-day old rat fetuses do not metabolize niclosamide (Andrews *et al.*, 1983).

Radiolabeled ethanolamine is rapidly taken up by cells, phosphorylated, and converted to phosphatidylethanolamine. After intraperitoneal administration in rats, >50% of the dose is found in liver, spleen, kidneys, heart, brain and diaphragm with 85% associated with the lipid fractions; 11.5% is exhaled via CO₂ (Knaak *et al.*, 1997). The rate limiting step in formation of phosphatidylethanolamine is formation of ethanolamine phosphate (Elder *et al.*, 1983) and thus incorporation of exogenous ethanolamine into phospholipids can be saturated.

4.2.4 Excretion

Excretion of ¹⁴C-niclosamide equivalents, niclosamide, and metabolites have been evaluated after oral administration in rats and humans. Human information can be found in Section 5.2.

When ¹⁴C-niclosamide ethanolamine salt was administered orally to male rats at 50 mg/kg, about one-third of the dose was absorbed from the gastrointestinal tract and eliminated in the urine within 24 hours; the remainder of the dose (two-thirds) was in feces (Andrews *et al.*, 1983, Duhm *et al.*, 1961). Similar absorption and excretion patterns were found when rats were given 50 mg/kg orally once daily for 7 days.

After a single oral dose of ¹⁴C-niclosamide to rats, approximately half of the radiolabel equivalents were excreted in urine and half in feces over a 6-day period (Griffiths 1979). The major excretory product in urine was the reduced 2,5'-dichloro-4'-amino-salicylanilide metabolite (31% of total dose) and related O- and N-glucuronide conjugates (14% of total dose); unchanged niclosamide in urine accounted for 5% of the total dose administered (Griffiths 1979). In feces, the major excretion product in rats was unchanged niclosamide (35% of total dose), with the 4'-nitro-reduced metabolite (2,5'-dichloro-4'-amino-salicylanilide) representing 10% of the total dose. A majority of niclosamide in feces was derived from biliary excretion where only niclosamide and niclosamide-O-glucuronide have been identified (Griffiths 1979).

4.3 Toxicology

The toxicity of niclosamide (free acid) and niclosamide ethanolamine has been evaluated in several studies published over the past 5 decades including single-dose and repeat-dose oral studies.

4.3.1 Single-Dose Studies

An overview of the available acute single-dose toxicity data in laboratory animals indicates low acute toxicity of niclosamide (free acid) and niclosamide ethanolamine via oral route of exposure (Table 4 and Table 5) and inhalation route (Table 6 and Table 7)

Further, ethanolamine administered orally as a single agent has a low order of acute toxicity with reported LD₅₀s of 700 to 15,000 mg/kg in mice, 1720 to 2750 mg/kg in rats, and 1000 to 2900 mg/kg in rabbits (Elder *et al.*, 1983; Knaak *et al.*, 1997).

Table 4: Single-Dose Oral Toxicity Studies with Niclosamide (free acid)

Species	Reported Dose	Effect	Source
Mouse	1500 mg/kg	LD50	Andrews 1983
Rat	> 5000 mg/kg	LD50	Andrews 1983; WHO 1988
Rat, female	4000 mg/kg	LD50	Awad 1995
Rat, male	> 3710 mg/kg	LD50	Andrews 1983
Rat, female	3710 mg/kg	LD50	Andrews 1983
Rat	> 1000 mg/kg	LD50 (no deaths or clinical signs)	EPA 1999
Rabbit	> 5000 mg/kg	LD50	Andrews 1983
Cat	> 1000 mg/kg	LD50	Andrews 1983
Dog	> 250 mg/kg	LD50	Andrews 1983

LD=lethal dose, IP= intraperitoneal, IV=intravenous.

Table 5: Single-Dose Oral Toxicity Studies with Niclosamide Ethanolamine

Species	Reported Dose	Effect	Source
Rat	> 10,000 mg/kg	LD50	Andrews 1983
Rat, female	> 5000 mg/kg	LD50	Andrews 1983
Rabbit	> 4000 mg/kg	LD50	Andrews 1983
Cat	> 500 mg/kg	LD50	Andrews 1983

LD=lethal dose, IP= intraperitoneal, IV=intravenous.

Table 6: Single-Dose Inhalation Toxicity with Niclosamide Ethanolamine Salt

Species	Reported Dose	Effect	Source
Rat, male and female	2270 mg/m ³ /hr	LD50	WHO 1988
Rat, male and female	>20,000 mg/m ³ /hr	LD50	Andrews 1983

Table 7: Single Dose Inhalation Toxicity with Niclosamide Ethanolamine Salt 70% Wettable Powder

Species	Reported Dose	Effect	Source
Rat, female and male	> 2260 mg/m ³ /4hr (700g/kg w.p.)	LD50	WHO 1988
Rat, male	> 1475 mg/m ³ /hr	LD50	Andrews 1983
Mouse, male	>1475 mg/m ³ /hr	LD50	Andrews 1983
Mouse, male	>1580 mg/m ³ /hr	LD50	Andrews 1983
Mouse, male	>1610 mg/m ³ /4hr	LD50	Andrews 1983
Rabbit	>1475 mg/m ³ /hr	LD50	Andrews 1983
Rabbit	<2420 mg/m ³ /4hr	LD50	Andrews 1983
Guinea-pig	<430 mg/m ³ /30min	LD50	Andrews 1983
Guinea-pig, male	<1000 mg/m ³ /20min	LD50	Andrews 1983
Guinea-pig, male	1000 mg/m ³ /10min	LD50	Andrews 1983
Guinea-pig, male	700 mg/m ³ /5min	LD50	Andrews 1983

Recently, another acute toxicity study has been performed by Costabile et al. (2015) in rats administered niclosamide as a bolus intratracheally. Twenty-four hours after in vivo administration, bronchoalveolar lavage was obtained for protein evaluation as an index of lung damage. This test did not reveal any significant difference in the samples from rats treated with the formulation at the equivalent niclosamide dose of 100 µg/rat. However, a significant increase in the number of macrophages and neutrophils was found at this dose, although no sign of lung injury was present (measured by iNOS and COX-2 expression). iNOS and COX-2 are both markers of lung injury induced by mechanical (i.e ventilation) or chemical (e.g. acids) stress (Bonnans et al., 2006, Robertson et al., 2012, Peng et al., 2004).

4.3.2 Repeat-Dose Studies

Repeat-dose toxicity studies have been conducted with niclosamide and niclosamide ethanolamine in rats, mice, hamsters, dogs, and cats (Table 8 and Table 9). Overall, these studies demonstrate that niclosamide ethanolamine in various formulations is well tolerated via oral administration. Systemic findings with niclosamide administered orally were only

seen after high repeat oral doses (5000 mg/kg/day) and included marginal decreases in hemoglobin concentration and erythrocyte count.

Limited data in rats and dogs are available on the oral repeated-dose toxicity of ethanolamine alone. In a 3-month toxicity study in rats (10/group) fed ethanolamine up to a high dose of 2670 mg/kg/day, increased liver and kidney weights were observed at ≥ 640 mg/kg/day and mortality and pathological changes were reported observed at 1250 mg/kg/day (specifics of the changes are not available). Beagle dogs (12/group) administered ethanolamine via diet for 2 years at dose levels up to 22 mg/kg/day in a proprietary cosmetic formulation had no observed changes in a number of parameters including clinical signs, body and organ weights, clinical pathology, and gross and microscopic pathology (Elder 1983). No microscopic findings were observed in the livers from pregnant rats administered up to 1000 mg/kg/day in the diet for up to at least 90 days (Fiume *et al.*, 2015).

Table 8: Repeat Oral (in diet) Toxicity Studies with Niclosamide or Niclosamide Ethanolamine

Species, Sex	Doses tested	Duration	Conclusion	Reference
Rat, M+F	10,000 or 25,000 ppm in diet (500 or 1250 mg/kg/day) (niclosamide ethanolamine)	326 or 319 days	Reduction in BW in males at both doses No effects on females	WHO 1988; Hayes 1991
Rat, M+F	up to 20,000 ppm in diet (~1610 mg/kg/day) (niclosamide)	14 weeks	NOAEL = 20,000 ppm	WHO 1988; Hayes 1991
Rat, M	1000 or 2500 mg/kg/day orally for 55 or 64 days, followed with 10,000 or 25,000 ppm in diet for 365-381 days (niclosamide)	55 or 64 days plus 365-381 days	Reduced BW in the HD group NOAEL = 1000 mg/kg/day (oral) + 10,000 ppm (in diet)	WHO 1988; Hayes 1991
Rat, M+F	300, 1250 or 5000 ppm in diet (30, 125 or 500 mg/kg/day) Bayer 73 (~70% niclosamide)	90 days	NOAEL = 5000 ppm	EPA 1999
Hamster, M+F	300, 1250 or 5000 ppm (39, 177 or 726 mg/kg/day) Bayer 73 (~70% niclosamide)	90 days	NOAEL = 1250 ppm (based on decreased body weight gain)	EPA 1999
Dog, M+F	62.5, 250 or 1000 ppm in diet (1.56, 6.25 or 25 mg/kg/day) Bayer 73 (~70% niclosamide)	180 days	NOAEL = 1000 ppm	EPA 1999

F=female, M=male, NOAEL= no observed adverse effect level

Table 9: Repeat Oral (gavage) Toxicity Study with Niclosamide or Niclosamide Ethanolamine

Species, Sex	Doses tested	Duration	GLP Status	Conclusion	Reference
Rat, M+F	up to 5000 mg/kg/day (niclosamide)	4 weeks	NC	Marginal decreases in hemoglobin concentration and erythrocyte count at 5000 mg/kg/day in both sexes. NOAEL = 2000 mg/kg/day	WHO 1988; 2002
Dog, M+F	up to 4500 and 6000 mg/day (niclosamide ethanolamine)	4 weeks	NC	NOAEL = 6000 mg/day	WHO 1988; Hayes 1991
Dog, M+F	100 mg/kg/day (capsules) (niclosamide ethanolamine)	1 year	NC	NOAEL = 100 mg/kg/day	WHO 1988; Hayes 1991
Dog, M+F	100 mg/kg/day (capsules) (niclosamide)	1 year	NC	NOAEL = 100 mg/kg/day	WHO 1988; Hayes 1991
Rabbit, M+F	100 mg/kg/day (niclosamide)	4 weeks	NC	NOAEL = 100 mg/kg/day	WHO 2002

F=female, male=M, NOAEL= no observed adverse effect level

4.3.2.1 Study ID: CRL-263283: Dose Range Finding and 2 Week Inhalation Toxicity Study in the Rat

The pivotal 2-week safety study in rats was assessed using daily dose levels of 15 and 50 mg/kg (10 rats/sex/group for main study evaluation); both vehicle and air control groups were also included. This pivotal phase was preceded by a rangefinding phase which selected a high dose level of 50 mg/kg for use in the pivotal 2-week phase. Microscopic evaluation of the nasal cavity in rats after 2 weeks of daily dosing revealed a non-adverse minimal hypertrophy of goblet (mucin-secreting) cells in the nasal septum/nasopharynx at 15 and 50 mg/kg which was not dose related; these changes were not observed in the vehicle or air control groups and were considered an adaptive change to repeated administration of niclosamide ethanolamine. At an estimated nasal deposition factor of 25%, body weight of 0.25 kg, and nasal surface area of 14 cm² (Gizurason 1989), the local dose to the nasal cavity at the highest no-observed-adverse-effect level (NOAEL) in rats is 223 µg/cm².

4.3.2.2 Study ID: CRL-263284: Dose Range Finding and 2 Week Inhalation Toxicity Study in the Beagle Dog

The pivotal 2-week safety study in dogs was assessed using daily dose levels of 2.5 and 4.37/4.16 mg/kg (3/sex/group for main study evaluation); both vehicle and air control groups were also included. This pivotal phase was preceded by a rangefinding phase which selected a high dose level of 8 mg/kg for use in the pivotal 2-week phase; however, the targeted high dose level administered was missed and only 4.37 or 4.16 mg/kg was delivered. In addition, 3 high-dose male dogs showed complications due to inhalation dosing after the first dose and were sacrificed early along with the remaining high-dose male dogs as a precaution. Microscopic evaluation of the nasal cavity in male and female dogs at 2.5 mg/kg and in females at 4.16 mg/kg after 2 weeks of daily dosing revealed no changes to the nasal cavity. In one of the early sacrificed males (sacrificed within 10 hours after first dose), a minimal erosion in the respiratory nasal cavity (Level II) with associated acute inflammation and may have been secondary to vomiting. In conclusion, at an estimated nasal deposition factor of 20%, body weight of 8.8 kg, and nasal surface area of 221 cm² (Gizurason 1989), the local dose to the nasal cavity at the highest 2-week no-observed-effect level (NOEL) in dogs is 33.0 µg/cm².

4.3.3 Genotoxicity

A considerable number of genotoxicity studies have been published on niclosamide ([Table 10](#)). Niclosamide was negative for forward mutations in the mouse lymphoma assay (Cifone 1995).

Mixed results have been found in the Ames test. These results could result from the antibacterial activity of niclosamide, which could lead to increased mutation rate due to cellular stress resulting from subinhibitory exposure to an antibiotic ([Table 10](#)).

In a mammalian cell cytogenetic assay (chromosome aberration in bone marrow cells), where male and female Crl:CD(ICR) BR mice, 15/sex/group, were exposed to niclosamide (98.9%) at either 1250, 2500 or 5000 mg/kg by a single oral gavage administration, no evidence was found of chromosome aberrations in bone marrow cells induced over background (Murli 1995).

An *in vivo* study showed an increase in sister chromatid exchange and in chromosome aberration at the highest tested dose given IP in mice (25 mg/kg); no evidence was found for genotoxicity when given orally (Giri *et al.*, 1996).

Prior published safety reviews concluded that “niclosamide seems to be free of relevant mutagenic effects in mammals” (Andrews *et al.*, 1983). These investigators highlighted that data must be evaluated in light of the low rate of absorption and the rapid elimination of niclosamide in mammals, which may explain the absence of mutagenic effects in animal

experiments except in a high-dose intraperitoneal dose in 1 study (Giri *et al.*, 1996). The same conclusion was drawn by the Environmental Protection Agency (EPA 1999), which concluded that niclosamide showed no evidence of causing mutagenicity.

Ethanolamine was negative for bacterial mutagenicity assays using the National Toxicology Program (NTP) protocols with 5 *Salmonella* strains and one *E. coli* strain with and without metabolic activation employing the preincubation method. Ethanolamine was also negative for chromosomal aberrations in rat liver cells and in the cell transformation assay using hamster embryo cells (Dean *et al.*, 1985; Mortelmans *et al.*, 1986).

An obscure report in Russian indicated weak mutagenic and clastogenic effects with ethanolamine but these were not corroborated using NTP protocols as noted above. But details are not available (Arutiunuan *et al.*, 1987; Knaak *et al.*, 1997).

Table 10: Genotoxicity Studies with Niclosamide

Assay, Species	Doses tested	Duration	GLP Status	Conclusion	Reference
Ames test, <i>S. typhimurium</i>	NC	NC	NC	Without metabolic activation: no mutagenic potential With metabolic activation: slight mutagenic effect	WHO 1988
Ames test, <i>S. typhimurium</i>	5-50 mg/mL	NC	NC	No mutagenic effect with and without S9	Macphee 1977
Ames test, <i>S. typhimurium</i> (TA1535, TA1537, TA1538, TA98, TA100)	1-50 µg/plate			Mutagenic in TA 98 and TA1538 (+S9)	Cortinas de Nava 1983
In vitro point mutation test, NC	NC	NC	NC	Reported negative, but unreliable due to unreported conditions	WHO 2002
In vitro mouse lymphoma assay	2.5 to 80 µg/mL		NC	No evidence of mutagenic potential (Study classified acceptable by US-EPA)	EPA 1999
In vivo chromosome aberration in BM cells, mice	1250, 2500 or 5000 mg/kg/day	Single gavage administration	NC	No evidence of chromosome aberrations (Study classified acceptable by US-EPA)	EPA 1999 Murli 1995
In vivo chromosome aberration in	6.25, 12.5 or 25 mg/kg/day	Single IP injection	NC	Chromosome aberrations at 25 mg/kg	Giri 1996

BM cells, mice					
In vivo micronucleus test, NC	NC	NC	NC	Not mutagenic, not clastogenic	WHO 2002
In vivo Sister Chromatic Exchange test, mice	6.25, 12.5 or 25 mg/kg/day	Single IP injection	NC	SCE at 25 mg/kg	Giri 1996
In vivo dominant lethal test, mouse	NC	NC	NC	No indication of mutagenic potential in F1 mice	WHO 1988

4.3.4 Reproductive Toxicity

Several studies have assessed the reproductive toxicity of niclosamide and have found that niclosamide does not show significant reproductive toxicity (Table 11). A document from WHO (WHO 1988) reported no embryotoxic or teratogenic effects after administration of 1000 mg/kg/day on gestation days 7–10, 10–12, or 13–16. No teratogenic or embryotoxic effects were seen in rats treated orally after administration of 1000 mg/kg/day on days 4–6, 7–9, or 10–12.

For ethanolamine alone, several embryo-fetal development studies and a combined fertility and peri-/postnatal development (2-generation) study have been reported. The rat oral gavage and feeding studies (Hellwig *et al.*, 1997; Fiume *et al.*, 2015), as well as rat and rabbit dermal developmental toxicity studies (Liberacki *et al.*, 1996), were intended to be safety evaluations and followed regulatory agency guidance for the conduct of reproductive and developmental toxicity studies and were conducted under Good Laboratory Practice (GLP) regulations. The overall conclusion of the reproductive and developmental studies is that ethanolamine does not cause embryotoxic, fetotoxic, or teratogenic effects and is not toxic to F1 or F2 generations at doses up to 450 mg/kg/day in the presence of maternal toxicity (e.g., decreased food consumption and lower body weight relative to control). Based on available data, the weight of evidence indicates that ethanolamine has a low potential for developmental effects at doses which are maternally toxic.

Table 11: Reproductive and Developmental Oral Toxicity Studies with Niclosamide

Species, Sex	Doses	Duration	Conclusion	Reference
Rat, F	80 mg/kg/day, oral (gavage) (niclosamide)	Days 5 to 15 pc Hysterectomy on Day 20 pc	No maternal toxicity Decreased fetal body weight Ossification delays No teratogenic potential	Awad 1995

Species, Sex	Doses	Duration	Conclusion	Reference
Rat, F	1000 mg/kg/day (niclosamide)	Days 4-6, 7-9 or 10-12 pc	No embryotoxic or teratogenic effects	Andrews 1983
Rabbit, F	1000 mg/kg/day (niclosamide)	Days 7-10, 10-12 and 13-16 pc	Acute toxicity in does No embryotoxic or teratogenic effects	Andrews 1983; WHO 2002
Rabbit, F	20, 60 and 180 mg/kg/day (Bayer 73, 70% niclosamide)_	Days 8-18 pc	No maternal toxicity LOAEL for maternal and developmental toxicity could not be established	EPA 1999
Rabbit, F	1000 mg/kg/day (niclosamide)	Days 4-6, 7-9 and 10-12 pc	Acute toxicity in does No embryotoxic or teratogenic effects	WHO 2002
Rabbit, F	up to 1500 mg/kg/day (niclosamide)	NC	No embryotoxic or teratogenic effects	WHO 2002

F=female, LOAEL=lowest-observed-adverse-effect level, NC=not communicated, pc=postcoitum (presence of sperm cells in a vaginal lavage or confirmed mating)

4.3.5 Phototoxicity

Phototoxicity of ATx201 has been evaluated both in vitro and in vivo (Table 12). A preliminary experiment demonstrated absorption of ATx201 in the UVA (315 to 400 nm) range, with an absorption maximum of 350 nm ($\epsilon=2.3 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) in PEG400. Based on this result, a GLP in vitro phototoxicity study (K69/JA/01) was conducted to screen for phototoxicity potential of ATx201 as per OECD 432 guidance. This study compared the reduction in viability (using neutral red update) of BALB/c 3T3 mouse fibroblasts exposed to ATx201 in the presence and absence of ultraviolet radiation (UVA, 365 nm).

ATx201 had a photoirritancy factor (PIF) of 9.02 and a mean photo effect (MPE) of 0.25. The positive control, Chlorpromazine, generated a PIF of 39.3 and MPE of 0.96. Compounds with a PIF value >5 and a MPE value >0.15 are considered potentially phototoxic (OECD 432 guidance).

Based on a positive result in the in vitro phototoxicity study, a GLP in vivo phototoxicity study in Crl:SKH1-hr hairless mice was conducted using the clinical ATx201 OINTMENT topical formulations 2% and 4%, as well as a gel placebo. Approximately 1 hour after topical application (T_{max} of ATx201 in epidermis), skin was exposed to UVA, UVB, and visible light from a xenon lamp; skin reactions were evaluated before and during the UV exposure for at least 1 and 4 hours after the exposure, and at least 1, 2, and 3 days after the UV exposure. There was no evidence of dermal phototoxicity elicited by UV exposure over the 3-day observation period with the placebo, 2%, or 4% ATx201 OINTMENT. Thus, the

clinical ATx201 OINTMENT formulations do not have the potential to induce phototoxicity when applied topically to the skin.

Table 12: Phototoxicity Studies

Species, Strain, Sex	Doses tested	GLP Status	Conclusion	Reference
In vitro using 3T3 fibroblast	Up to 60µg/mL ± UV exposure	Yes	Positive for phototoxicity potential	K69/JA/01
Mouse, Crl:SKH1-hr hairless, female	Clinical 2% and 4% OINTMENTS and a placebo ± UV exposure	Yes	Negative for phototoxicity	20137005

4.3.6 Carcinogenicity

Two carcinogenicity studies conducted with niclosamide ethanolamine have been published by the National Cancer Institute (NCI) (Table 13) and showed no evidence of carcinogenicity in mice and rats. Based on these results, niclosamide ethanolamine (clonitralid) has been classified as a non-carcinogenic.

Table 13: Carcinogenicity Studies with 78-Week Niclosamide Ethanolamine

Species, Strain, Sex	Doses tested	Conclusion	Reference
Rat, Osborne-Mendel, male and female	14,216-28,433 ppm (711-1421 mg/kg/day)	No carcinomas (Conducted under the National Cancer Institute auspices)	NCI 1978
Mouse, B6C3F1, male and female	274-549 ppm (39-78 mg/kg/day)	Males: poor survival rate did not permit evaluation of carcinogenic potential. Females: no carcinomas (Conducted under the National Cancer Institute auspices)	NCI 1978

4.4 Nonclinical Assessment of Safety

The nonclinical safety of Niclosamide (given as free acid or ethanolamine salt) after oral and dermal application is well characterized through the previous extensive nonclinical safety evaluation including studies to evaluate single and repeat-dose toxicity, reproductive and developmental effects, phototoxicity, genotoxicity, and carcinogenicity.

As reported in the literature, the NOAEL dose in rats via oral route is up to 1250 mg/kg/day for niclosamide ethanolamine over 11 months and 1000 mg/kg/day for niclosamide free acid over 12 months. The NOAEL in dogs was the highest dose tested of 100 mg/kg/day with either dose form administered orally over 12 months.

Microscopic evaluation of rat and dog nasal cavities after inhalation exposure revealed no safety concerns.

Based on the observed intranasal safety in the 2-week rat and dog studies and, as reported in the literature, the chronic oral toxicity studies in rats and dogs up to 12 months, the following safety margins were derived to support the proposed dosing regimen in PROTECT:

Systemic and local intranasal safety margins against planned human dose

Species	Safety margin against planned human dose of 5.6 mg/day or 0.09 mg/kg/day ^a	
	Systemic Oral Safety Margin [mg/kg]	Local Intranasal Safety Margin [mg/cm ²]
Rat	11,111-fold ^b	6.4-fold ^c
Dog	1111 -fold ^b	0.9-fold ^d

^a 5.6 mg niclosamide ethanolamine salt corresponds to 0.09 mg/kg, assuming a 60 kg adult. For local exposure of 5.6 mg, it is assumed a nasal surface area of 160 cm² and a 100% deposition factor which equates to 35.0 µg/cm².

^b Systemic safety margins are based on NOAEL reported for chronic oral toxicity studies.

^c Calculations based on the highest 2-week inhalation rat dose of 50 mg/kg (local NOAEL), the mean body weight reported in the on-going inhalation study of 0.25 kg, nasal cavity deposition factor of 25%, and a nasal surface area of 14 cm² in the rat (Acta Pharmaceutica Nordica, 1990) which equates to 223 µg/cm².

^d Calculations based on the highest 2-week inhalation dog dose of 4.16 mg/kg (local NOEL), the mean body weight reported in the study of 8.8 kg, nasal cavity deposition factor of 20%, and a nasal surface area of 221 cm² in the dog (Acta Pharmaceutica Nordica, 1990) which equates to 33.0 µg/cm².

Overall, the proposed dosing regimen for the PROTECT study can be supported by the nonclinical study results available for UNI911, niclosamide free acid, and/or niclosamide ethanolamine.

5 EFFECTS IN HUMANS

5.1 Introduction

Even though no clinical trials with UNI911 have yet been conducted in humans for SARS-CoV-2, substantial preclinical and clinical data are published supporting the safety of niclosamide after oral and topical application.

The main findings of these are summarized in the Summary of Product Characteristics (SmPC) of Yomesan®, chewable tablets, one of the niclosamide products marketed by Bayer (Appendix 1).

5.2 Investigational Studies

Moreover, five clinical trials have been completed by UNION investigating the safety and efficacy of ATx201 as topical application. The key characteristics of the trial and the key safety findings are summarized in [Table 14](#) below:

Table 14: Summary of investigational studies conducted by UNION

UNION Study Code	Number of subjects	Treatment duration	Strength of formulation	Key Safety finding
ATx201-001	30 healthy volunteers	7 days	2%	No signs of irritation or other safety signals observed
ATx201-006	35 healthy volunteers	21 days, qd	2% and 4%	No signs of irritation
ATx201-005	240 subjects	24 days	2% and 4%	No signs of sensitization
ATx201-002	36 AD subjects	7 days, qd or bid	2%	A PK analysis showed that systemic concentration 1 hour after the last application was a mean of 1.64 ng/mL in the twice-daily group, while the once-daily group had a systemic level of 0.789 ng/mL. No safety signals observed
ATx201-003	31 AD subjects	3 weeks	2%	No significant safety signals observed
ATx201-004	210 Impetigo subjects	≤5 day	2% and 4%	No significant safety signals observed

5.3 Pharmacokinetics and Product Metabolism in Humans

5.3.1 Oral Studies from Literature

Two studies in the literature offer PK information following oral administration of niclosamide, alone or in combination therapy.

In one study, the following metabolites were excreted in the form of glucuronides following oral administration of niclosamide: niclosamide, 2',5-dichloro-4'-aminosalicylanilide (indicating a metabolic reduction of the 4'-nitro moiety to an amino group) and 2',5-dichloro-4'-acetaminosalicylanilide (Duhm *et al.*, 1961).

In another study, between 2% and 25% of 2 g of ¹⁴C-labeled niclosamide given orally was eliminated in the urine over a 4-day period; the remainder was eliminated in feces. Elimination of ¹⁴C-niclosamide equivalents was essentially complete after 1 to 2 days and maximal serum concentration equivalent was 250 to 6000 ng/mL (Andrews *et al.*, 1983). After oral administration, niclosamide is only partially absorbed from the intestinal tract and the absorbed fraction is rapidly eliminated by the kidneys.

5.3.2 Metabolism

Hydroxylation and glucuronidation of niclosamide have been observed using human liver microsomes and individually expressed human cytochrome P450 enzymes and UDP-glucuronosyltransferases. The majority of the hydroxylation and glucuronidation activity was mediated by CYP1A2 and UGT1A1, respectively (Lu *et al.*, 2015).

5.3.3 Pharmacokinetic Drug Interactions

Niclosamide is a substrate and an inhibitor of CYP1A2 (Lu *et al.*, 2015, Bapiro *et al.*, 2001). The K_i for CYP1A2 using phenacetin as substrate is 2.7 μM or 883 ng/mL (Bapiro *et al.*, 2001) and accordingly potential drug interactions may occur with other substrates of CYP1A2. In addition, niclosamide was shown to be both an inhibitor and activator of CYP2C9, depending on substrate (Bapiro *et al.*, 2001; Egnell *et al.*, 2003). For example, niclosamide had an apparent activation (400%) of 7-methoxy-4-trifluoromethylcoumarin demethylase activity and inhibition (K_i = 6.00 μM or 1962 ng/mL) of diclofenac 4-hydroxylase activity. Another report indicated niclosamide to be a potential weak inhibitor of CYP2D6 (Masimirembwa *et al.*, 1995).

Studies have demonstrated that niclosamide is not an *in vitro* inhibitor of either P-glycoprotein or multidrug resistance-associated protein (MRP1), but may be a potential weak inhibitor of breast cancer resistance protein (BCRP) (Ivnitski-Steele *et al.*, 2008; Strouse *et al.*, 2013).

5.4 Marketing Experience for Oral Form of Niclosamide

Introduced in the early 1960s and primarily used as an anthelmintic drug, oral niclosamide is currently marketed in developing countries, including South Africa, and in several European countries, including Finland, France, Germany, Netherlands, and Sweden under the trade names Yomesan, Kontal, and Tredemine for treatment of children and adults.

Adverse effects of orally administered niclosamide, reviewed by Ofori-Adjei *et al.* (2008), and mentioned in product insert labels, include gastrointestinal disorders such as nausea, vomiting, stomach and abdominal pain and diarrhea, lightheadedness, allergic and hypersensitivity reactions such as pruritus, erythema or rash, cyanosis, and anaphylactic reactions (WHO Model Formulary 2008, Ofori-Adjei *et al.*, 2008). From 1971 to 2004, there have been 82 reports from 16 countries involving 173 suspected adverse drug reactions to niclosamide in the WHO database. The most common adverse reactions are those involving the skin and appendages (43), gastrointestinal tract (38) and cardiovascular system (28). There were 9 reports of anaphylactic shock and anaphylactoid reactions (Uppsala Monitoring Centre database, accessed January 2015). It is now known that those allergic reaction are related to the release of parasite antigens from dead tapeworm and that they are not indicative of an idiosyncratic reaction.

6 SUMMARY OF DATA AND GUIDANCE FOR THE INVESTIGATOR

Niclosamide is approved and marketed for the oral treatment of tapeworm infections in adults and children > 2 years of age for more than 40 years. Its safety profile is well established and mainly characterized by gastro-intestinal side-effects.

UNI911 Nasal Spray Solution 1% developed by UNION has besides its pleiotropic activities potent anti-SARS-CoV-2 properties, making UNI911 a promising candidate as a prophylactic treatment for patients at particularly high risk of COVID-19 and its complications.. Niclosamide has been identified as the leading candidate for activity against SARS-CoV-2 in two separate library screens of existing approved drugs (Giacca *et al*; 2020. Unpublished data).

Recently, Niclosamide has shown potent eradication of SARS-CoV-2 in vitro (Jeon 2020): among 50 drugs evaluated, Niclosamide exhibited a strong antiviral effect with an IC₅₀ at 0.28 µM when the IC₅₀ of chloroquine, lopinavir, and remdesivir used as reference drugs had IC₅₀ values of 9.12, 7.28, and 11.41 µM, respectively. In addition to this anti-viral activity, Niclosamide was also shown to have antibacterial and anti-inflammatory effect in vivo (Cabrita *et al.*, 2019, Tharmalingam *et al.*, 2018). Interestingly, niclosamide has shown in a mouse model that it inhibits mucus production and cytokine release and induces bronchorelaxation.

Given that niclosamide has a poor bioavailability following oral administration, Union has initiated the development of topical form of this compound. A first product developed for the topical treatment of atopic dermatitis has confirmed the good safety profile of such approach with no potential for inducing sensitization and very low irritation profile of concentrations up to 4%. Particularly interesting and more relevant to the new proposed indication, is an irritation study conducted on abraded skin (with stratum corneum removed with tape striping) where niclosamide at 4% was less irritant than the negative control.

The new formulation described in this brochure, UNI911 Nasal Spray Solution 1%, allow deposition of the drug to the nasal cavity, given that this anatomical zone is a major area for viral replication.

6.1 Reference safety Information

No SARs are considered expected by the sponsor for the purpose of expedited reporting of SUSARs and identification of SUSARs in the “Cumulative summary tabulation of serious adverse reactions” in the DSUR for the IMP.

6.2 Dose rationale

The proposed dosing of 140 uL UNI911 to each nostril will provide a combined dose of 280 uL 1% niclosamide ethanolamine solution. With an assumption of 90 % protein binding and a 2 hour half life, the suggested twice daily dosing would result in a free concentration of niclosamide that at peak concentration would be 857-fold higher than the IC90 and after 6 half lifes (corresponding to the minimum exposure with the twice daily regimen) would be 13-fold higher than the IC90. Based on these assumptions the proposed highest feasible and well tolerated dose of UNI911 should lead to a continuous maintenance of a concentration at least 13-fold above the IC90 in the nasal epithelium, which based on the available antiviral efficacy against SARS-CoV-2 would limit the ability the virus to establish in the nasal cavity and accordingly act as a prophylactic treatment against COVID19.

With regard to potential systemic exposure after intranasal application, the twice daily administration of 140 µL per nostril, i.e. 560 µL per day), corresponds to a dose of 5.6 mg of niclosamide ethanolamine salt per day (= 4.7 mg niclosamide free acid, as niclosamide ethanolamine salt contains 84% niclosamide). Even in a highly conservative approach, where 100% of the administered dose would be assumed to permeate and reach blood circulation, the maximal systemic exposure would still be approx. 43 times lower than the one reported with oral niclosamide at the approved dose (Yomesan chewable tablets) of 2g/day (based on the reported 10% bioavailability of oral niclosamide by Chang et al. 2006, i.e. $200 \text{ mg day}^{-1} / 4.7 \text{ mg day}^{-1} = 43$). Systemic toxicity observed in chronic oral studies in rat and dogs generated safety margins that are 11,111 -fold (rat) and 1111-fold (dog) greater than the proposed clinical intranasal dose based on body weight.

In addition, nonclinical studies in rats and dogs, indicated no adverse safety signals with regard to local effects in the nasal cavity.

Furthermore, the irritation potential of niclosamide (4% ointment) was investigated on abraded skin for 21 days in healthy human volunteers with no signs of irritation (see Section 1.1). Abraded skin is considered a robust model for potential irritative effects on mucosa in other tissues. This is because abraded skin is characterized by the loss of stratum corneum and hence the epithelial cells of the skin will be directly exposed to the drug. As the major cell type in the nasal cavity are epithelial cells similar to the skin epithelial cells,

we believe that the results of the irritation study support the safety of UNI911 when applied to the respiratory mucous membrane, including the one covering the nasal cavity

Use of cyclodextrin as a solubilizer

The proposed formulation of the drug product to be tested in the proposed study contains 15 % cyclodextrin (HP- β -Cyclodextrin).

A risk assessment for cyclodextrins conducted by the EMA concluded that a dose “below 20 mg/kg/day of cyclodextrin no serious adverse effects are to be expected for all routes of administration and no statement deemed necessary” (EMA/CHMP/333892/2013, 2017). As we are planning to use 15% of HP β CD in a 140 μ L solution per nostril twice daily in the proposed solution, the planned dose of HP β CD would correspond to 84 mg/day ((140 μ L x 4 x 15%) = 84 mg/day) in the proposed study (assuming 100% exposure), which is 14.3 times lower than the threshold defined by EMA, assuming a 60 kg adult (20 mg/kg/day x 60 kg = 1200 mg/day; 1200 mg/day / 84 mg/day = 14.3).

6.3 Contraindications

Niclosamide is not approved for use in children < 2 years of age, and during breastfeeding. If treatment during pregnancy is urgently needed, this should be done only after the second trimester. Moreover, the effects on breast milk and unique use in the elderly are unknown.

UNI911 is contraindicated in subjects allergic to niclosamide or its derivatives.

6.4 Interactions

Given the expected low systemic exposure of niclosamide following inhalation, the risk for drug interactions is considered low. However, no DDI study following inhalation was conducted in this program and that as a matter of precaution, administration of UNI911 should be made separately from any other drug administration.

6.5 Use during Pregnancy and Lactation

The use in pregnancy is insufficiently studied. There are no adequate animal studies regarding the effects on pregnancy, embryonal / fetal development, birth and postnatal development. The potential risk for humans is unknown.

UNION is not aware of any data regarding distribution of niclosamide into breast-milk. As a precaution, women with known, suspected, or planned pregnancy, and lactating mothers should be excluded from clinical trials with UNI911.

6.6 Undesirable Effects

Though no clinical trials have yet been conducted in humans for COVID-19, the Summary of Product Characteristics (SmPC) of Yomesan®, one of the niclosamide products marketed by Bayer, lists the adverse reactions below, which are based on spontaneous reports. Frequencies can therefore not be stated and are classified as not known:

- *Immune system disorders*
Allergic reaction (eg erythema, pruritus and rash), anaphylactic reaction and anaphylactic shock²
- *Nervous system disorders*
Dizziness (dizziness)
- *Angiopathy*
Cyanosis
- *Diseases of the gastrointestinal tract*
Nausea, gastrointestinal pain, abdominal pain, gagging, diarrhea
- *Skin and subcutaneous tissue disorders*
Skin rash, pruritus, hyperhidrosis
- *General disorders and administration site conditions*
Malaise (fatigue)

Side effects sometimes reported with nebulization procedures include bronchospasm and coughing. Symptomatic treatments usually recommended for the management of such events, if severe or persistent, include beta-mimetics and lidocaine.

6.7 Overdose

No cases of accidental or deliberate overdosage with niclosamide are known.

7 REFERENCES

References are available on request.

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² These reactions have been linked to release of dead tapeworm antigens and are hence not idiosyncratic

Acta Pharmaceutica Nordica 2: 105-22, 1990

Al-Gareeb AIA, Gorial FI, Mahmood AS. Niclosamide as an adjuvant to etanercept in treatment patients with active rheumatoid arthritis: an 8-week randomized controlled pilot study. *Clin Rheumatol*. 2018 doi: 10.1007/s10067-018-4164-5.

Al-Gareeb, Ali I., Khalid D. Aljubory, and Hayder M. Alkuraishy. "Niclosamide as an anti-obesity drug: an experimental study." *Eating and Weight Disorders-Studies on Anorexia, Bulimia and Obesity* 22.2 (2017): 339-344.

Andrews P, Thyssen J, Lorke D. The biology and toxicology of molluscicides, Bayluscide. *Pharmacol Ther*. 1983;19(2):245–295.

Arutiunian, R. M., et al. "Mutagenic action of latex polymerization stabilizers in various test systems." *TSitologiia i genetika* 1987, 21.6: 450-454.

Awad OM. Assessment of the developmental toxicity of in utero exposure of Wistar Albino Rats to ametryne and niclosamide. *Pest Biochem Physiol*. 1995;53(1);1-9.

Bapiro TE, Egnell AC, Hasler JA, Masimirembwa CM. Application of higher throughput screening (HTS) inhibition assays to evaluate the interaction of antiparasitic drugs with cytochrome P450s. *Drug Metab Dispos*. 2001 Jan;29(1):30–5.

Bonnans, C., Fukunaga, K., Levy, M. A., & Levy, B. D. (2006). Lipoxin A4 regulates bronchial epithelial cell responses to acid injury. *The American journal of pathology*, 168(4), 1064-1072

Burock S, Daum S, Keilholz U, Neumann K, Walther W, Stein U. Phase II trial to investigate the safety and efficacy of orally applied niclosamide in patients with metachronous or synchronous metastases of a colorectal cancer progressing after therapy: the NIKOLO trial. *BMC Cancer*. 2018 Mar 15;18(1):297.

Cabrita, Inês, et al. "Niclosamide repurposed for the treatment of inflammatory airway disease." *JCI insight* 2019, 4.15.

ChemIDplus dataset on niclosamide [Internet]. Bethesda, MD. U.S. National Library of Medicine; Accessed on 26 Feb 2019. Available from: <https://chem.nlm.nih.gov/chemidplus/rn/50-65-7>.

Chang, Y-W, T-K Yeg, K-T Lim K-T, W-C Chen, H-T Yao, S-J Lan, Y-S Wu, H-P Hsieh, C-M Chen, C-T Chen. Pharmacokinetics of Anti-SARS-CoV Agent Niclosamide and Its Analogs in Rats. *Journal of Food and Drug Analysis*, 2006;14(4):329-333.

Cheong, Hyun-Ah, and Hoo-Kyun Choi. "Enhanced percutaneous absorption of piroxicam via salt formation with ethanolamines." *Pharmaceutical research* 19.9 (2002): 1375-1380.

Cifone M. Mutagenicity Test on Niclosamide in the L5178Y TK+/Mouse Lymphoma Forward Mutation Assay: Final Report: Lab Project Number: 16403-0-431: 20989: 431. 1995. Unpublished study prepared by Corning Hazleton Inc. 71 p.

Costabile, G., d'Angelo, I., Rampioni, G., Bondi, R., Pompili, B., Ascenzioni, F., ... & Quaglia, F. (2015). Toward repositioning niclosamide for antivirulence therapy of

Pseudomonas aeruginosa lung infections: development of inhalable formulations through nanosuspension technology. *Molecular pharmaceutics*, 12(8), 2604-2617.

Cortinas de Nava C, Espinosa J, García L, Zapata AM, Martínez E. Mutagenicity of antiamebic and anthelmintic drugs in the *Salmonella typhimurium* microsomal test system. *Mutat Res/Genet Toxicol*. 1983;117(1-2):79-91.

Dean, B.J., Brooks, T.M., Hodson-Walker, G. and Hutson, D.H. (1985). Genetic toxicology testing of 41 industrial chemicals. *Mutat. Res.* 153, 57-77.

Duhm B, Maul W, Medenwald H, Patzschke K, and Weber LA. Radioaktive Untersuchungen mit einem neuen Molluscidid. *Zeitschrift für Naturforschung*. 1961;16b:509-515.

Egnell, Ann-Charlotte, et al. "Generation and evaluation of a CYP2C9 heteroactivation pharmacophore." *Journal of Pharmacology and Experimental Therapeutics* 2003,307.3: 878-887.

EMA/CHMP/333892/2013, 2017

Elder, R. L., ed. 1983. Final report on the safety assessment of Triethanolamine, Diethanolamine, and Monoethanolamine. *J. Am. Coll. Toxicol.* 2:183-235.

EPA 738-R-99-007. Reregistration eligibility decision (RED) 3-Trifluoro-Methyl-4-Nitro-Phenol and Niclosamide. EPA-738-R-99-007. U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Washington, DC. 1999.

Fachinformation Yomesan® https://s3.eu-central-1.amazonaws.com/prod-cerebro-ifap/media_all/60387.pdf

Fan X, Li H, Ding X, Zhang QY. Contributions of Hepatic and Intestinal Metabolism to the Disposition of Niclosamide, a Repurposed Drug with Poor Bioavailability. *Drug Metab Dispos.* 2019;47(7):756-763.

Fenner A. Prostate cancer: niclosamide jumps the hurdle of enzalutamide resistance. *Nat Rev Urol.* 2014;11(8)424.

Fiume, Monice M., et al. "Safety Assessment of alkyl esters as used in cosmetics." *International journal of toxicology* 34.2_suppl 2015: 5S-69S.

Frayha GJ, Smyth JD, Gobert JG, Savel J. The mechanisms of action of antiprotozoal and anthelmintic drugs in man. *Gen Pharmacol.* 1997 Feb;28(2):273-99.

Giri AK, Adhikari N, Khan KA. Comparative genotoxicity of six salicylic acid derivatives in bone marrow cells of mice. *Mutat Res.* 1996;370(1):1-9.

Gizurarson S. *Acta Pharmaceutica Nordica*, 31 Dec 1989, 2(2):105-122

Glick GD and Luigi F. "Methods and compositions for treating conditions associated with an abnormal inflammatory responses." U.S. Patent Application No. 15/255,102. (WO 2-017/040864A1)

Goennert R, Results of laboratory and field trials with the molluscicide Bayer 73., *Bull. World Health Organ.* **25**, 483–501 (1961).

Grasselli, Giacomo, Antonio Pesenti, and Maurizio Cecconi. "Critical Care Utilization for the COVID-19 Outbreak in Lombardy, Italy: Early Experience and Forecast During an Emergency Response." *JAMA* (2020).

Griffiths LA, Facchini V. The major metabolites of niclosamide identification by mass spectrometry. A. Friferio (ed). *Recent Developments in Mass Spectrometry and Medicine*; Plenum Press, New York, 1979.

Guo, Jingjing, et al. "Niclosamide piperazine prevents high-fat diet-induced obesity and diabetic symptoms in mice." *Eating and Weight Disorders-Studies on Anorexia, Bulimia and Obesity* 2019, 24.1: 91-96.

Gupta, Deepak, et al. "Salts of therapeutic agents: chemical, physicochemical, and biological considerations." *Molecules* 2018, 23.7:1719.

Hayes, W.J., Jr., E.R. Laws, Jr., (eds.). *Handbook of Pesticide Toxicology. Volume 3. Classes of Pesticides*. New York, NY: Academic Press, Inc., 1991., p. 1496-1499.

Hellwig, J., and A. B. Liberacki. "Evaluation of the pre-, peri-, and postnatal toxicity of monoethanolamine in rats following repeated oral administration during organogenesis." *Fundamental and Applied Toxicology* 40.1 (1997): 158-162.

Ivnitski-Steele I, Larson RS, Lovato DM, Khawaja HM, Winter SS, Oprea TI, Sklar LA, Edwards BS. High-throughput flow cytometry to detect selective inhibitors of ABCB1, ABCC1, and ABCG2 transporters. *Assay Drug Dev Technol.* 2008;6(2):263-76.

Jeon, Sangeun, et al. "Identification of antiviral drug candidates against SARS-CoV-2 from FDA-approved drugs." *bioRxiv* 2020.

Jung, E., Nam, S., Oh, H., Jun, S., Ro, H. J., Kim, B., ... & Go, Y. Y. Neutralization of Acidic Intracellular Vesicles by Niclosamide Inhibits Multiple Steps of the Dengue Virus Life Cycle In Vitro. *Scientific reports*, 2019; 9 (1), 1-12.

Jurgeit A, McDEowell R, Moese S, Meldrum E, Schwendener, Greber U. Niclosamide is a proton carrier and targets acidic endosomes with broad antiviral effects. *PLoS Pathog.* 2012 Oct 25;8(10):e1002976.

Kadri H, Lambourne OA, Mehellou Y. Niclosamide, a drug with many (re)purposes. *ChemMedChem* [Internet]. 2018 Mar 30; Available from: <http://www.ncbi.nlm.nih.gov/pubmed/29603892%0Ahttp://doi.wiley.com/10.1002/cmdc.201800100>.

Knaak, J. B., et al. "Toxicology of mono-, di-, and triethanolamine." *Reviews of environmental contamination and toxicology*. Springer, New York, NY, 1997. 1-86.

Klonne, D. R., et al. "Two-week aerosol inhalation study on polyethylene glycol (PEG) 3350 in F-344 rats." *Drug and chemical toxicology* 12.1 (1989): 39-48.

Li, Rui, You S, Hu Z, Chen ZG, Sica GL, Khuri FR, Curran WJ, Shin DM, Deng X. Inhibition of STAT3 by niclosamide synergizes with erlotinib against head and neck cancer. *PloS One*. 2013 Sep 3;8(9):e74670.

Li, Yonghe, et al. "Multi-targeted therapy of cancer by niclosamide: A new application for an old drug." *Cancer letters* 349.1 (2014): 8-14.

Liang L, Huang M, Xiao Y, Zen S, Lao M, Zou Y, et al. Inhibitory effects of niclosamide on inflammation and migration of fibroblast-like synoviocytes from patients with rheumatoid arthritis. *Inflammation Research*. 2015;64(3-4), 225-33.

Liberacki, A. B., et al. "Evaluation of the developmental toxicity of dermally applied monoethanolamine in rats and rabbits." *Toxicological Sciences* 1996, 31.1: 117-123.

Lu D. Metabolism of the anthelmintic drug niclosamide by cytochrome P450 enzymes and UDP-glucuronosyltransferases: metabolite elucidation and main contributions from CYP1A2 and UGT1A1. *Xenobiotica*. 2015. doi: 10.3109/00498254.2015.1047812.

MacLean JA II, Sekulovski N, Bheemireddy H, Okuda H, Plunkett KN, Yu Z, Matzuk MM, Hayashi K. Post-transcriptional regulation by FXR1 and IGF2BP2 can be new insights and potential druggable mechanisms in ovarian cancer [abstract]. 51st Annual Meeting of the Society for the Study of Reproduction. 2018 Jul 13.

Macphee DG, Podger DM. Mutagenicity tests on anthelmintics: Microsomal activation of vipryinium embonate to a mutagen. *Mutat Res*. 1977;48(3-4):07-312.

Madrid PB, Panchal RG, Warren TK, Shurtleff AC, Endsley AN, Green CE, Andrey Kolokoltsov A, Davey R, Manger ID, Gilfillan K, Bavari S, Tanga MJ. Evaluation of Ebola Virus Inhibitors for Drug Repurposing. *ACS Infect Dis*. 2015;1(7):317-26.

Maltas E. Binding interactions of niclosamide with serum proteins. *J Food Drug Anal*. 2014;22(4):549-555

Marrugal-lorenzo JA, Serna-gallego A, Berastegui-cabrera J, Pachón J, Sánchez-Céspedes J. Repositioning salicylanilide anthelmintic drugs to treat adenovirus infections. *Sci Rep*. 2019;1-10.

Masimirembwa CM, Hasler JA, Johansson I. Inhibitory effects of antiparasitic drugs on cytochrome P450 2D6. *Eur J Clin Pharmacol*. 1995;48(1):35-8.

Mazzon, M., Ortega-Prieto, A. M., Imrie, D., Luft, C., Hess, L., Czieso, S., ... & Wright, E. (2019). Identification of broad-spectrum antiviral compounds by targeting viral entry. *Viruses*, 11(2), 176.

Morin F, Kavian N, Nicco C, Cerles O, Chéreau C, Batteux F. Improvement of Sclerodermatous Graft-Versus-Host Disease in Mice by Niclosamide. *J Invest Dermatol*. 2016a;136(11):2158-67.

Morin F, Kavian N, Nicco C, Cerles O, Chéreau C, Batteux F. Niclosamide Prevents Systemic Sclerosis in a Reactive Oxygen Species-Induced Mouse Model. *J Immunol* [Internet]. 2016b;197(8):3018-28. Available from: <http://www.jimmunol.org/lookup/doi/10.4049/jimmunol.1502482>.

Mortelmans, K. Haworth, S., Lawlor, T., Speck, W., Trainer, B. and Ziegler, E. (1986). Salmonella mutagenicity tests:2. Results from testing of 270 chemicals. *Environ. Mutagen.* 8, 1-119.

Murli H. Mutagenicity test on niclosamide. (2',5-Dichloro-4'-Nitrosalicylanilide) measuring chromosomal aberrations in vivo in mouse bone marrow cells: final report: lab project number: 16403-0-451: 22202: 451. Unpublished study prepared by Hazleton Washington, Inc. 1995;70 p.

Niazi, Safaraz K. Handbook of pharmaceutical manufacturing formulations: sterile products. CRC press, 2016.

Novel, Coronavirus Pneumonia Emergency Response Epidemiology. "The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19) in China." *Zhonghua liu xing bing xue za zhi= Zhonghua liuxingbingxue zazhi* 41.2 (2020): 145.

NCI (National Cancer Institute) U.S. Department of Health, Education, and Welfare Publication No. (NIH) 78-1341. Report on the Bioassay of Clonitralid for Possible Carcinogenicity. Technical Report No. 91. 1978.

Ofori-Adjei D, Dodoo ANO, Appiah-Danquah A, Couper M. A review of the safety of niclosamide, pyrantel, triclabendazole and oxfamiquine. *Intl J Risk Saf Med.* 2008;20(3):113–122.

Park, Jeong Su, Yu Seol Lee, and Soo Han Bae. "Repositioning of niclosamide ethanolamine (NEN), an anthelmintic drug, for the treatment of lipotoxicity." *Free Radical Biology and Medicine* 137 (2019): 143-157.

Peng, X., Abdulnour, R. E. E., Sammani, S., Ma, S. F., Han, E. J., Hasan, E. J., ... & Hassoun, P. M. (2005). Inducible nitric oxide synthase contributes to ventilator-induced lung injury. *American journal of respiratory and critical care medicine*, 172(4), 470-479.

Pearson RD, Hewlett EL. Niclosamide therapy for tapeworm infections. *Ann Intern Med.* 1985;102(4):550-551.

Ren X, Duan L, He Q, Zhang Z, Zhou Y, Wu D, Pan J, Pei D, Ding K. Identification of Niclosamide as a New Small-Molecule Inhibitor of the STAT3 Signaling Pathway. *ACS Med Chem Lett.* 2010;1,454–459.

Rothan, Hussin A., and Siddappa N. Byrareddy. "The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak." *Journal of Autoimmunity* (2020): 102433.

Robertson, J. A., Sauer, D., Gold, J. A., & Nonas, S. A. (2012). The Role of Cyclooxygenase-2 in Mechanical Ventilation–Induced Lung Injury. *American journal of respiratory cell and molecular biology*, 47(3), 387-394.

Ruan, Qiurong, et al. "Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China." *Intensive Care Medicine* 2020: 1-3.

Saiz JC, Martin-Acebes MA. Zika virus: a race in search for antivirals. *AAC*. 2017;24:61(6).

Schweizer MT, Haugk K, McKiernan JS, Gulati R, Cheng HH, Maes JL, et al. A phase I study of niclosamide in combination with enzalutamide in men with castration-resistant prostate cancer. *PLoS ONE*. 2018;13(6): e0198389. <https://doi.org/10.1371/journal.pone.0198389>

Sekulovski N, Whorton AE, Shi M, MacLean JAIL, Hayashi K. Endometriotic inflammatory microenvironment induced by macrophages can be targeted by niclosamide. *Biol Reprod*. 2019 Feb 1;100(2):398-408.

Shi L, Zheng H, Hu W, Zhou B, Dai X, Zhang Y, Liu Z, Wu X, Zhao C, Liang G. Niclosamide inhibition of STAT3 synergizes with erlotinib in human colon cancer. *Oncotargets Ther*. 2017 Mar 23;10:1767-76. doi:10.2147/OTT.S129449.

Shi, Y., Wang, Y., Shao, C. et al. COVID-19 infection: the perspectives on immune responses. *Cell Death Differ* 2020.

Shivaji U, Jeffery L, Batis N, Iacucci M, Ghosh S. P096 The anti-inflammatory effects of niclosamide on cytokines produced by PBMCs derived from IBD patients. *J Crohns Colitis*. 2019;13:S134-S134. 10.1093/ecco-jcc/jjy222.220.

Stachulski, A. V., Pidathala, C., Row, E. C., Sharma, R., Berry, N. G., Lawrenson, A. S., ... & Edwards, G. Thiazolides as novel antiviral agents. 2. Inhibition of hepatitis C virus replication. *Journal of medicinal chemistry*, 2011; 54(24), 8670-8680.

Strouse JJ, Ivnitski-Steele I, Waller A, Young SM, Perez D, Evangelisti AM, Ursu O, Bologa CG, Carter MB, Salas VM, Tegos G, Larson RS, Oprea TI, Edwards BS, Sklar LA. Fluorescent substrates for flow cytometric evaluation of efflux inhibition in ABCB1, ABCC1, and ABCG2 transporters. *Anal Biochem*. 2013;437(1):77-87.

Swan GE. The pharmacology of halogenated salicylanilides and their anthelmintic use in animals. *J S Afr Vet Assoc*. 1999;70(2):61-70.

Tai, Wanbo, et al. "Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine." *Cellular & Molecular Immunology* 2020: 1-8.

Tharmalingam N, Port J, Castillo D, Mylonakis E. Repurposing the anthelmintic drug niclosamide to combat *Helicobacter pylori*. *Sci Rep*. 2018;8(1):3701.

Vingsbo Lundberg C, Frimodt-Møller N. Efficacy of topical and systemic antibiotic treatment of methicillin-resistant *Staphylococcus aureus* in a murine superficial skin wound infection model. *Int J Antimicrob Agents*. 2013 Sep;42(3):272-5. doi: 10.1016/j.ijantimicag.2013.05.008. Epub 2013 Jul 06.

Wang YM, Lu JW, Lin CC, Chin YF, Wu TY, Lin LI, Lai ZZ, Kuo SC, Ho YJ. Antiviral activities of niclosamide and nitazoxanide against chikungunya virus entry and transmission. *Antiviral Res*. 2016 Nov;135:81-90.

Weinbach EC, Garbus J. Mechanism of action of reagents that uncouple oxidative phosphorylation. *Nature*. 1969;221:1016-18.

World Health Organization. Data sheet on pesticides No. 63: Niclosamide. WHO/VBC/88.63, March 1988.

World Health Organization. Food and Agriculture Organization World Health Organization. WHO specifications and evaluations for public health pesticides: Niclosamide. World Health Organization, Geneva. 2002. Accessed 30 May 2017 <http://www.who.int/whopes/quality/en/Niclosamide.pdf?ua=1>.

World Health Organization. WHO Model List. Essential Medicines 2005. Accessed 25 February 2020

https://apps.who.int/iris/bitstream/handle/10665/69097/a87351_fre.pdf?sequence=1

Wu CH, Li YR, Chen JJW, Chen YC, Chu CL, Pan LH, Wuh YS, Lin CC. Antihelminthic niclosamide modulates dendritic cells activation and function. *Cell Immunol*. 2014;288:15–23.

Wu, C. J., Jan, J. T., Chen, C. M., Hsieh, H. P., Hwang, D. R., Liu, H. W., ... & Lin, R. K. Inhibition of severe acute respiratory syndrome coronavirus replication by niclosamide. *Antimicrobial agents and chemotherapy*, 2004; 48(7), 2693-2696

Xu M, Lee EM, Wen Z, Cheng Y, Huang WK, Qian X, Tcw J, Kouznetsova J, Ogden SC, Hammack C, Jacob F, Nguyen HN, Itkin M, Hanna C, Shinn P, Allen C, Michael SG, Simeonov A, Huang W, Christian KM, Goate A, Brennand KJ, Huang R, Xia M, Ming GL, Zheng W, Song H, Tang H. Identification of small-molecule inhibitors of Zika virus infection and induced neural cell death via a drug repurposing screen. *Nat Med*. 2016 Oct;22(10):1101–7.

Xu, Jimin, et al. "Broad Spectrum Antiviral Agent Niclosamide and Its Therapeutic Potential." *ACS Infectious Diseases* 2020.

Ye Y, Zhang X, Zhang T, Wang H, Wu B. Design and evaluation of injectable niclosamide nanocrystals prepared by wet media milling technique. *Drug Dev Ind Pharm*. 2015;41(9):1416-24.

Zhang LX, Zhao HJ, Sun DL, Gao SL, Zhang HM, Ding XG. Niclosamide attenuates inflammatory cytokines via the autophagy pathway leading to improved outcomes in renal ischemia/reperfusion injury. *Mol Med Rep*. 2017 Aug;16(2):1810-16.

Zhou, Fei, et al. "Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study." *The Lancet*. 2020.

APPENDIX 1

SMPC Yomesan® – This is a translation of the german document “Fachinformation Yomesan®”

1. NAME OF THE DRUG

Yomesan®

500 mg chewable tablets

2. QUALITATIVE AND QUANTITATIVE COMPOSITION

Each chewable tablet contains 500 mg niclosamide.

For full list of ingredients, see section 6.1.

3. FORM OF ADMINISTRATION

Chewable Tablets

Gray-yellow, round chewable tablets with the mark "FE" on one side and the "Bayer Cross" on the other side.

4. CLINICAL INFORMATION

4.1 Applications

Infections through

- Taenia saginata (bovine tapeworm),
- Taenia solium (pork tapeworm),
- Diphylobothrium latum (fish tapeworm),
- Hymenolepis nana (dwarf tapeworm).

Yomesan eradicates the intestinal tapeworms. Therefore, no effect can be expected in cysticercosis or echinococcosis caused by cystic larvae (fins) of T. solium, E. multilocularis or E. granulosus residing in extraintestinal tissue.

4.2 Dosage and Administration

Dosage

Unless prescribed otherwise, the following dosages apply:

Infections with	Patient group	Intake of chewable tablet, Day 1	Per Day, Day 2 to 7
Taenia saginata, Taenia solium, Diphylobothrium latum	Children 6 years and older and adults	4 chewable tablets	Omitted
	Children from 2 to 6 years old	2 chewable tablets	Omitted
	Children under 2 years of age	1 chewable tablet	Omitted
Hymenolepis nana	Children 6 years and older and adults	4 chewable tablets	2 chewable tablets per day

	Children from 2 to 6 years old	2 chewable tablets	1 chewable tablet per day
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Limited kidney function

Niclosamide is not substantially absorbed by the gastrointestinal tract and does not affect renal function. Consequently, a dose adjustment for patients with impaired renal function is not required.

Limited liver function

Niclosamide is not substantially absorbed by the gastrointestinal tract and does not affect hepatic function. Consequently, dose adjustment is not required with impaired liver function.

Route of administration

For oral intake. The daily dose is taken at once after breakfast. The tasty chewable tablets must be chewed thoroughly to a fine pulp and then washed down with little water. They can also be taken decomposed in water.

Children

For smaller children, the chewable tablets are conveniently grounded to a fine pulp before given to the child with little water.

General suggestions

The elimination of the intestinal mucus abundantly produced in a tapeworm infection can be promoted by administering acidic fruit juices; The worms, which are settled under the mucus deposits, are thus easier to reach for the drug.

In principle, if constipation is present the digestive system should be emptied prior to treatment with Yomesan. Additional dietary actions are not required. If, following therapy, the tapeworm is to be expelled quickly and preferably in one piece, a saline laxative (eg Glauber's salt, Epsom salt) which leads to diarrheal stools and washing out of the parasite may be given at the earliest 2 hours after chewing tablet intake (or in the case of *Hymenolepis nana* after the last dose). Due to digestion it is possible that the tapeworm head is not always found in the stool even after drastic purgation. If not purged, the tapeworm will appear in parts over the following days. In case of infections with the pork tapeworm (*Taenia solium*) drastic purging should not be renounced.

4.3 Contraindications

- Hypersensitivity to the active substance or to any of the ingredients listed in section 6.1
- Breastfeeding (see section 4.6)

4.4 Special warnings and precautions for use

In pork tapeworm infections (*Taenia solium*), there is a risk of cysticercosis. To prevent this, a drastic purgation is recommended aiming to eliminate the lower tapeworms containing mature eggs as quickly as possible. This prevents that in case of uncleanness any released eggs at the defecation will be later transferred from the finger of the patient into the mouth, which can cause cysticercosis.

If not drastically purged after Yomesan treatment, it is possible that single tapeworm remnants appear in the stool until about 2 days after the treatment. Later, no tapeworm limbs or tapeworm eggs should be found in the stool. New infections with *Taenia saginata* and *Taenia solium* should lead to the occurrence of tapeworm limbs or tapeworm eggs in the stool only after 3 months.

Only with *Hymenolepis nana*, the control period is about 14 days, as surviving scolices regenerate very rapidly to the sexually mature tapeworm and therefore tapeworm eggs can be excreted with the stool again after 10 days.

4.5 Interaction with other medicinal products and other forms of interaction

Niclosamide is soluble in alcohol, which can lead to increased absorption. Therefore, simultaneous consumption of alcohol should be avoided.

4.6 Fertility, pregnancy and lactation

Pregnancy

The use of Yomesan in pregnancy is insufficiently studied. There are no adequate animal studies regarding the effects on pregnancy, embryonal / fetal development, birth and postnatal development (see section 5.3). The potential risk for humans is unknown.

Therefore, if possible, treatment with Yomesan should be started after giving birth. If treatment during pregnancy is urgently needed, this should be done only after the second trimester.

Lactation

Since it is not known whether niclosamide passes into the breast milk, breast-feeding is not recommended.

Fertility

There are no clinical or preclinical *in vivo* data about a potential effect of niclosamide on fertility.

4.7 Effects on ability to drive and use of machines

This drug may impair the ability to drive or operate machinery as a result of reactions of the central nervous system (see also section 4.8)

4.8 Side effects

The adverse reactions listed below are based on spontaneous reports. Frequencies can therefore not be stated and are classified as not known (frequency can not be estimated based on available data).

Immune system disorders

Allergic reaction (eg erythema, pruritus and rash), anaphylactic reaction and anaphylactic shock

Nervous system disorders

Dizziness (dizziness)

Angiopathy

Cyanosis

Diseases of the gastrointestinal tract

Nausea, gastrointestinal pain, abdominal pain, gagging, diarrhea

Skin and subcutaneous tissue disorders Skin rash, pruritus, hyperhidrosis
General disorders and administration site conditions Malaise (fatigue)

Report of suspected adverse reactions

Reporting suspected adverse reactions after approval is of great importance. It allows continuous monitoring of the benefit-risk balance of the drug. Healthcare professionals are encouraged to report any suspected adverse reactions to the Federal Institute for Drugs and Medical Devices, Dept. Pharmacovigilance, Kurt-Georg-Kiesinger-Allee 3, D-53175 Bonn, website: <http://www.bfarm.de>.

4.9 Overdose

No cases of overdoses were reported.

5. PHARMACOLOGICAL PROPERTIES

5.1 Pharmacodynamic properties

Pharmacotherapeutic group: salicylic acid derivatives, ATC code: P02DA01;
Anthelmintic mechanism of action

Niclosamide acts locally through direct contact with the tapeworm's scolex. Micronised crystals increase the contact surface and ensure special effectiveness.

Niclosamide inhibits oxidative phosphorylation in mitochondria of the parasite. This leads to the death of the scolex and connected limbs. The chain of body segments thus loses its stability and get eliminated with the defecation in the whole or in individual smaller parts.

5.2 Pharmacokinetic properties

After oral administration of niclosamide, between 2-25% of the administered dose was detected in the urine, which can be considered as the minimum level of absorption. The maximum plasma concentrations were 0.2-6 µg / ml.

5.3 Preclinical safety data

Based on the results of toxicological studies on acute and chronic toxicity, no risk to humans is expected if the prescribed dose range is complied to.

Under conditions of clinical use, mutagenic effects of Yomesan are not expected.

There are no studies on the carcinogenic potential of Yomesan.

In a reproductive toxicology study in which rats were treated with 2.5 times of the human therapeutic dose throughout organogenesis, the number of dead and malformed fetuses was increased and the weight of the female fetuses was decreased.

Treatment of rats with the 25-fold human therapeutic dose over a period of 3 days during organogenesis did not induce embryo / fetotoxic effects.

6. PHARMACEUTICAL PARTICULARS

6.1 List of excipients

Magnesium stearate, corn starch, sodium lauryl sulphate, povidone K25, sodium saccharin 2 H₂O, talc, vanillin

6.2 Incompatibilities

Not applicable.

6.3 Storage life

5 years

6.4 Special precautions for storage

This drug does not require special storage conditions.

6.5 Type and contents of container

Outer carton with aluminum / aluminum blister pack of 4 chewable tablets

6.6 Special precautions for disposal and other handling

No special requirements.

7. OWNER OF THE APPROVAL

Bayer Vital GmbH

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8. APPROVAL NUMBER

6245026.00.00

9. DATE OF APPROVAL/ EXTENSION OF THE APPROVAL

Date of approval / Date of last renewal of the authorization: 19 August 2005

10. STATUS OF INFORMATION

06/2014

11. DISTRIBUTION

Pharmacy-only