2.4. NONCLINICAL OVERVIEW

TABLE OF CONTENTS

ABBREVIATIONS / GLOSSARY

Term	Explanation
bw	Body weight
CHO	Chinese hamster ovary
Cmax	Maximum plasma concentration
CoNS	Coagulase-negative staphylococci
DNA	Deoxyribonucleic acid
Eur. Ph.	European Pharmacopoeia
HEX	Hexamidine
HEX D	Hexamidine diisethionate
Ki	Inhibitory constant
LD50	Lethal dose 50 per cent
MIC	Minimum inhibitory concentration
MRT	Mean residence time
NOEL	No Observed Effect Level
NOS	Nitric oxide synthase
PHMB	Polyhexamethylene biguanide
TDLo	Lowest published toxic doses

LIST OF FIGURES AND TABLES

2.4.1 OVERVIEW OF THE NONCLINICAL TESTING STRATEGY

2.4.1.1 INTRODUCTION

This dossier concerns a hybrid application according to the Article 10 (3) of the EC Directive 2001/83/EC to request the marketing authorisation for T1680 (hexamidine diisethionate 0.1%) eye drops solution. T1680 contains the same active pharmaceutical ingredient and excipients in the same concentrations as the currently authorised product, Desomedine® registered by Bausch & Lomb for the single unit dose (reference product). The T1680 solution was formulated as sterile eye drops packaged in single dose units (SDU). The therapeutic indications and posology recommended for T1680 are the same as Desomedine®.

T1680 is a topical antiseptic, indicated:

- for the treatment of:
  - purulent bacterial conjunctivitis caused by susceptible microorganisms
  - keratoconjunctivitis
  - blepharitis
  - chronic tear duct infections
- as a preoperative antisepsis for the conjunctival sacs

The recommended dose is one drop into the conjunctival sac of the affected eye(s), 4 to 6 times daily. The total duration of treatment should not exceed 8 days to avoid the emergence of resistant strains.

Hexamidine diisethionate, a diamidine, has been used in medicine as an antiseptic for over half a century [Grare et al, 2010]. It is a hydrosoluble cationic agent with antimicrobial activity against bacteria, fungi, yeasts, and free-living amebae [Grare et al, 2010, Aimard et al, 1998].

The proposed medicinal product T1680 is a sterile preservative-free solution for ophthalmic use containing 1 mg/mL hexamidine diisethionate. The complete formula is as follows:

Table 2.4.1.1-1: Composition of T1680

Name of the ingredients	**Formula** g / 100 mL	**Function**	**Reference to standards**
Hexamidine diisethionate	0.100	Active substance	Current Eur. Ph. [0549]
Borax	[redacted]	Buffer agent	Current Eur. Ph. [0013]
Boric acid		Buffer agent	Current Eur. Ph. [0001]
Sodium chloride		Isotonizing agent	Current Eur. Ph. [0193]
Water for injections		Vehicle	Current Eur. Ph. [0169]

All ingredients are listed in the European Pharmacopoeia.

2.4.1.2 NATURE OF THE REQUEST

This dossier concerns a hybrid application for a marketing authorization for T1680 ophthalmic solution. T1680 contains the same active substance hexamidine diisethionate and excipients in the same concentrations as the currently authorised product Desomedine®.

Ophthalmic formulations containing hexamidine diisethionate are available for numerous years as medicinal products, registered and marketed in various European countries for more than 20 years.

2.4.1.3 APPLICANT'S PROPOSAL AND SUPPORTING DATA

This nonclinical overview discusses the preclinical pharmacological and toxicological aspects of hexamidine diisethionate with particular reference to its ophthalmic use.

Therefore, a literature review has been carried out by the Applicant. A search on PubMed and on toxicological databases (such as RTECS, ECHA, ATSDR) was performed and relevant articles published until September 2020 including hexamidine (diisethionate) and specific issues in animals such as pharmacodynamics/pharmacokinetics/toxicological aspects in the title and abstract, were selected then analyzed. The sources of published information mainly included peer-reviewed journal articles and the quality of the data is therefore considered satisfactory.

2.4.2 PHARMACOLOGY

2.4.2.1 PRIMARY PHARMACODYNAMICS

2.4.2.1.1 General pharmacology

Hexamidine (HEX) is a strong organic base and is an aromatic diamidine. Diamidines are well known for their antimicrobial effects resulting from the cationic surface-active properties generated from the bipolar structures of the molecules [Perrine et al, 1995].

Hexamidine was first synthesized as the dihydrochloride salt in the late 1930s but, subsequently, the diisethionate salt was preferred for use, presumably because of its more favourable water solubility. Today it is the diisethionate form which is used in medicinal and care products [Parisi et al, 2017]. The active substance of T1680 is hexamidine diisethionate (HEX D).

The structure of hexamidine diisethionate is shown in Figure below.

Figure 2.4.2.1-1: Structure of hexamidine diisethionate

HEX D has been used in medicine as an antiseptic for over half a century [Grare et al, 2010].

In line with the broad spectrum of antimicrobial activity shown by hexamidine in its various forms, the European Union Cosmetics Directive 76/768/EEC, Annex VI, allows HEX D as a preservative for cosmetics and toiletries up to a maximum concentration of 0.10% [CIR, 2007].

It is a hydrosoluble cationic agent with antimicrobial activity against bacteria, fungi, yeasts, and free-living amebae [Grare et al, 2010, Aimard et al, 1998]. This positively charged molecule binds with high affinity to the negatively charged cell walls and membranes of bacteria, thus causing disruption of the target cell by perturbation of the binding sites [Grare et al, 2010].

HEX was initially developed as a trypanocidal agent. The anti-protozoal activity of hexamidine was further explored more than 50 years later when Brasseur et al. [Brasseur et al, 1994] successfully used HEX D to treat two subjects affected by *Acanthamoeba* keratitis.

An *in vitro* study from Perrine et al. [Perrine et al, 1995] showed that HEX D was effective not only against *Acanthamoeba* trophozoites but also against the dormant cyst forms. Bailly et al. [Bailly et al, 1997] thus hypothesized that the amoebicidal activity of hexamidine might have been directly related to its capacity to selectively bind DNA.

Antimicrobial activity

In terms of antibacterial properties, HEX D has been reported to be effective against *Pseudomonas aeruginosa*, *Proteus*, *Escherichia coli*, *Staphylococcus aureus* and *Tsukamurella paurometabolum* [van Ketel, 1975, Granel et al, 1996].

The efficacy of HEX D against a series of multidrug-resistant gram-positive bacteria has been demonstrated [Grare et al, 2010]. These authors tested the *in vitro* activity of hexamidine diisethionate against 39 multidrug-resistant Gram-positive bacteria (15 *Staphylococcus aureus*, 12 coagulase-negative staphylococci, and 14 *Enterococcus* spp.) and 30 multidrug resistant Gram-negative bacteria (20 *Enterobacteriaceae* and 10 nonfermenting bacilli).

The *in vitro* activities of hexamidine against *Staphylococcus aureus*, coagulase-negative staphylococci (CoNS), and *Enterococcus* spp., with various resistance phenotypes are reported in the following table.

Table 2.4.2.1-1: *In vitro* activities of hexamidine against *Staphylococcus aureus*, coagulase-negative staphylococci (CoNS), and *Enterococcus* spp. [Grare et al, 2010**]**

The *in vitro* activities of hexamidine against *Enterobacteriaceae*, with various resistance phenotypes are reported in the following table.

Table 2.4.2.1-2: *In vitro* activities of hexamidine against *Enterobacteriaceae*, with various resistance phenotypes [Grare et al, 2010**]**

The *in vitro* activities of hexamidine against non-fermenting bacilli, with various resistance phenotypes are reported in the following table.

Table 2.4.2.1-3: *In vitro* activities of hexamidine against non-fermenting bacilli, with various resistance phenotypes [Grare et al, 2010**]**

(): number of strains tested.

2.4.2.1.2 Ocular pharmacology – Mechanism of action

2.4.2.1.2.1 Literature data

Ophthalmic preparations containing hexamidine diisethionate 0.1% are currently commercially available for the treatment of minor eye infections, such as conjunctivitis and blepharitis.

Staphylococcus aureus, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa* are common causes of eye infection. Actually, *Staphylococcus epidermidis* is the organism most commonly isolated from eyes with post-operative endophthalmitis. Furthermore, Pseudomonas is the most frequent etiologic agent of contact lens—associated microbial keratitis, being responsible for up to 2/3 of cases. Fungi, including *Candida*, account for more than 50% of all culture-proven keratitis cases in tropical and subtropical regions and more than 50% of all cases of endogenous endophthalmitis [Review in Pinna et al, 2020].

In a recent study [Pinna et al, 2020] hexamidine diisethionate solution showed rapid *in vitro* antimicrobial activity against *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Candida* species but was poorly active against *Pseudomonas aeruginosa*. These results confirm that hexamidine diisethionate remains an efficient antimicrobial agent for common eye infections.

Results of this study of the *in vitro* antimicrobial activity of an ophthalmic solution containing hexamidine diisethionate 0.05% [Pinna et al, 2020] are detailed hereafter

The ability of the ophthalmic solution containing hexamidine diisethionate 0.05% to kill the organisms tested at different exposure times is shown in Table below.

Table 2.4.2.1-4: Microbial Growth at Different Times After Exposure to an Ophthalmic Solution Containing Hexamidine Diisethionate 0.05% [Pinna et al, 2020]

After 1-minute incubation, there was no growth on the plates seeded with *Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus* clinical isolate, *Staphylococcus epidermidis* clinical isolate, and all 5 Candida species tested. Conversely, the ophthalmic solution failed to kill both the clinical isolate and the ATCC reference strain of *Pseudomonas aeruginosa* after 30 minutes exposure and needed 24 hours to eradicate the organisms. Positive controls consistently showed growth at all exposure times. No growth was observed in the plates seeded with the negative control.

The ophthalmic solution containing hexamidine diisethionate showed a good, rapid antimicrobial activity against 5 clinical Candida isolates and multiresistant strains of *S. aureus* and *S. epidermidis*. Conversely, the hexamidine solution was not so rapidly effective against a clinical isolate and an ATCC reference strain of *P. aeruginosa*, taking more than 30 minutes to eradicate the organisms. These results are consistent with the more former study performed by Grare et al [Grare et al, 2010] where Hexamidine diisethionate was found to show moderate antibacterial activity against Gram-positive organisms but was poorly active against *Pseudomonas aeruginosa*.

The antimicrobial activity of Hexamidine is optimum at pH 5 to 9 and may be inactivated by chloride or sulfate ions and some proteins [Hill,1995 cited in CIR, 2007].

Hexamidine has also been found to be active on *Acanthamoeba castellanii* [Taravaud et al, 2017]. In the 1990s, the efficacy of HEX as an amoebicidal agent was demonstrated in a number of studies [Brasseur et al, 1994; Perrine et al, 1995]. The lethal effects of hexamidine as the other diamidines result from interactions of the protonated amidine groups attached to each benzene ring with the amphipathic lipids of the plasma membrane bilayer of amoebae, inducing structural changes that lead to modifications of cell permeability which could be responsible for the leakage of ions, water, and various biomolecules [Perrine et al, 1995].

HEX D in combination with polyhexamethylene biguanide (PHMB) was found active on abscess development in the rat model of chronic amoebic keratitis [Vasseneix et al, 2006]. In this study, thirty rats optimally infected and betamethasone treated (10^4 parasites and 0.28 mg/week, respectively) were divided into 4 groups of 6 rats, topically treated with PHMB 0.02% (group 1), HEX D 0.1% (group 2), a combination of PHMB 0.02%, and HEX D 0.1% (group 3), or miltefosine, 0.01% (group 4), respectively. The untreated group consisted of 5 animals (group 5). Corneal treatment was initiated 6 days after parasite inoculation and first betamethasone injection. Agents were administered as eyedrops 3 times a day for 21 days.

Results showed no difference between groups in the time intervals of abscess appearance ($p = 0.3$). At the end of the study, the ratio of rats with corneal abscesses was lower in the group treated with PHMB and HEX D ($p = 0.015$) than in all other treated and untreated groups ($p > 0.05$). In cultures, A. *polyphaga* was grown from samples from 4 of 6, 1 of 6, 1 of 6, 4 of 6, and 1 of 5 rats in groups 1, 2, 3, 4, and 5, respectively.

Anti-*A. polyphaga* activities of 5 concentrations of PHMB, HEX D, their combination, and miltefosine were evaluated *in vitro*. Hexamidine diisethionate exhibited MIC = 555 µM, and both PHMB and miltefosine displayed a better efficacy (MIC = 14 and 6 µM, respectively). The highest antiamoebal effect was obtained with a combination of PHMB and HEX D, which was synergistic (Fractional inhibitory concentration FIC = 0.06) [Vasseneix et al, 2006].

In addition, the combination of PHMB and HEX D exerted a synergistic effect *in vivo*, resulting in corneal healing 12 days after inoculation and was more effective than PHMB, HEX D, or miltefosine alone [Vasseneix et al, 2006].

2.4.2.1.2.2 In vitro study performed with HEXAMIDINE GILBERT 0.1%, single-dose eye drops (same formulation as T1680)

An *in vitro* study has been conducted by the previous Marketing Authorization Holder to compare the antibacterial activity of the generic medicinal product HEXAMIDINE GILBERT® 0.1% single-dose eye drops, which has the same formulation as T1680, and the reference product DESOMEDINE® Eye drops [Dusart G. Hexamidine: comparative study of the antibacterial activity of two eye drops, 10/24/2000].

The Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) were determined for the two products on a panel of 50 strains. These strains included collection strains (n=6) and wild strains isolated at the Montpellier University Hospital - France (n=44).

These were mainly Gram-positive strains (20 *Staphylococcus* including 10 *Staphylococcus aureus*, 10 *Enterococcus - Streptococcus* strains, mainly *E. faecalis* strains). Gram-negative bacteria were also tested (10 strains of *Enterobacter*, 5 strains of *Acinetabacter* and 5 strains of *Haemophilus*).

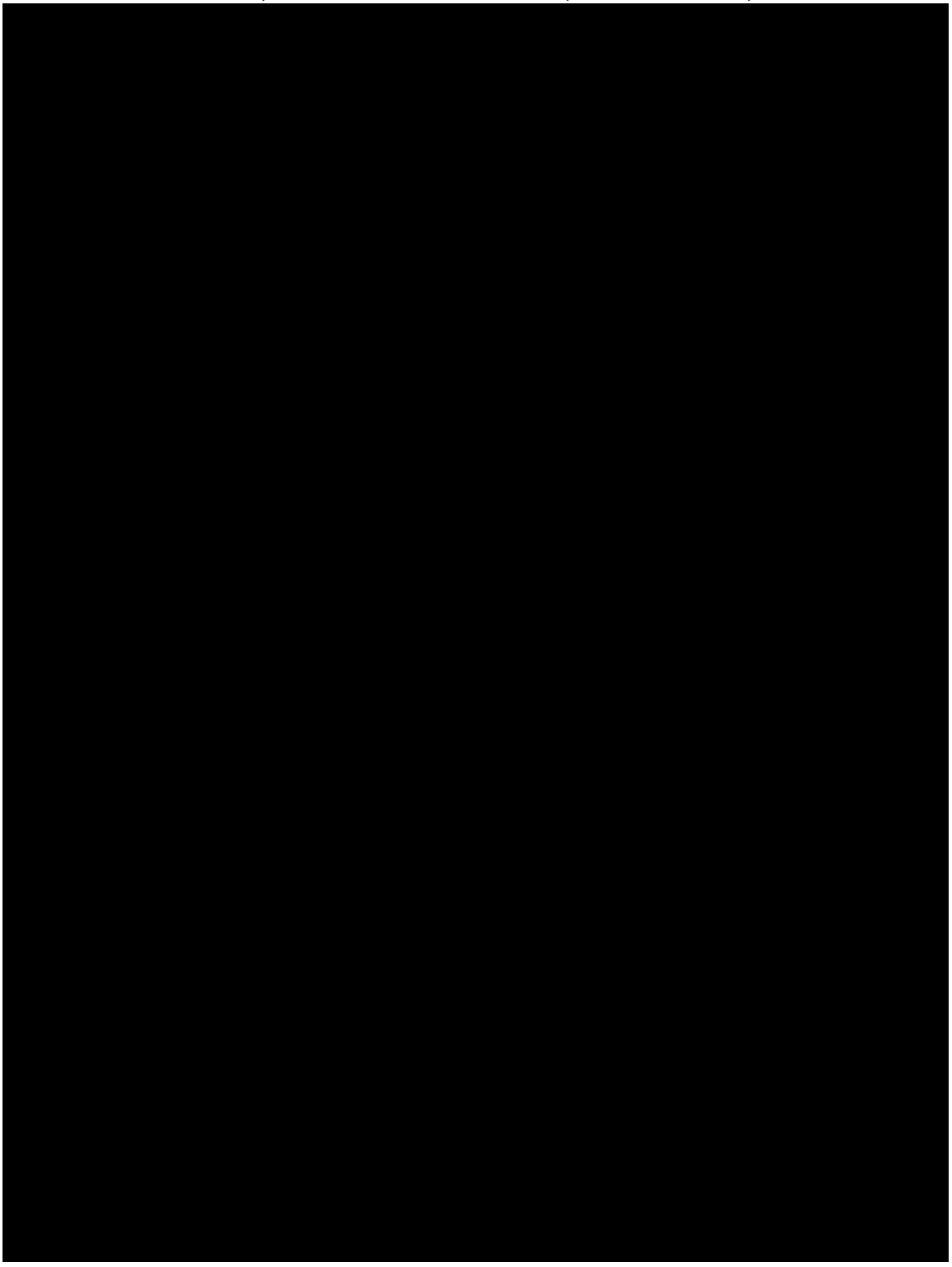
The wild strains tested in this study were chosen because of their involvement in various ocular pathologies.

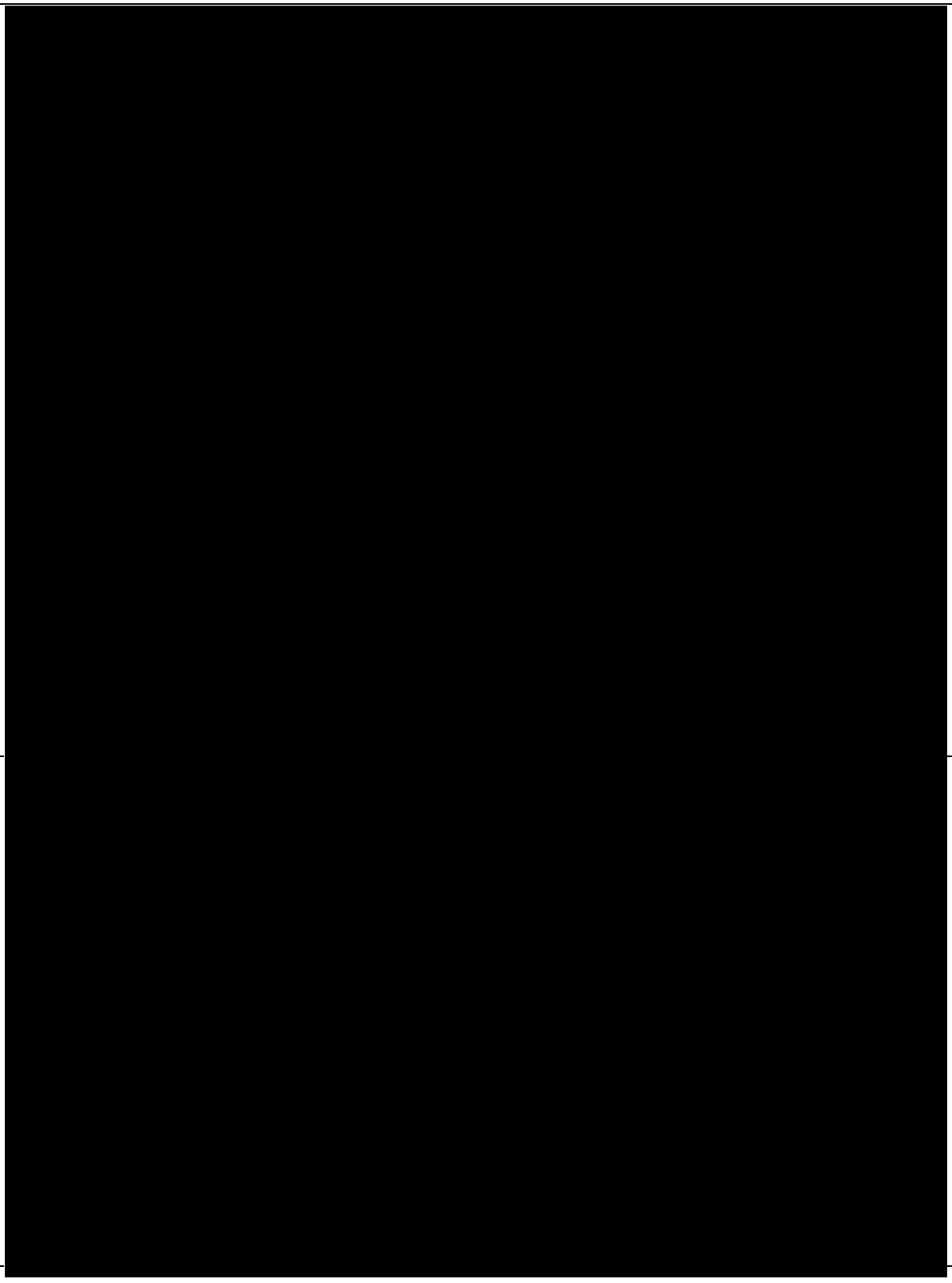
The MICs were studied by the technique of serial dilutions in liquid medium, in microplates, using successive dilutions of each of the products at 0.1% hexamidine diisetionate, *i.e.* 1000 mg/L (500 - 250 - 125 - 62 , 5 - 31.2 - 15.6 - 7.8 - 3.9 - 1.9 - 0.95 and 0.47 mg/L). Before reading, the microplates were incubated at 35-37°C for 24 to 48 hours under aerobic conditions.

The MBCs were determined by culture on agar after reading the MICs and counting the surviving bacteria.

The MIC and MBC values for the Test product and the Reference product are reported in the following Table.

Table 2.4.2.1-5: Comparative antibacterial activity of the Test product (same formulation as T1680) and the Reference Product (DESOMEDINE®)





These results showed that the antibacterial activities of both products were comparable. In the very few cases of discrepancy, the values obtained differed only by one dilution, a difference which can be attributed to the sensitivity of the method.

The best bacteriostatic and bactericidal activities were obtained against Gram-positive bacteria, and particularly against strains of *Staphylococci*. The MIC values were 0.47 mg/L and 0.95 mg/L for the most sensitive strains and 7.8 mg/L for the most resistant strains.

The values of MBC obtained on the strains of *Staphylococci* showed a good bactericidal activity, since they were equal to those of the MICs or they differed from them by only three dilutions (*S. aureus* n ° 13 and 23).

Regarding *Enterococci*, the MIC values were homogeneous and equal to 3.9 mg/L, except for the *E. hirae* ATCC 10541 strain (1.9 mg/L). The MBC values differed from those of the MICs by 3 to 4 dilutions, demonstrating a more moderate bactericidal activity than that observed with *Staphylococci*.

Regarding Gram-negative bacteria, the activity of the two products was more variable depending on the species, with MIC values ranging from 0.95 mg/L to 250 mg/L. For MBCs, values ranged from 1.9 to 250 mg/L.

2.4.2.1.2.3 Mechanism of action

The exact mechanism of diamidine antibacterial efficacy, including HEX D, is still unclear. However, due to its native positive charge, it is thought that HEX D binds with high affinity to the negatively charged cell walls and membranes of bacteria and that disruption is brought about by perturbations of these binding sites resulting in inhibition of oxygen uptake and induced leakage of amino acids. In this sense, HEX D might be considered to be acting as a cationic surface-active agent [McDonnell and Russell, 1999].

2.4.2.2 SECONDARY PHARMACODYNAMICS

In addition to biocidal activity, HEX and other diamidines have demonstrated enzyme inhibition properties. Upregulation of the major cholesterol and fatty acid uptake pathways has also been demonstrated in a skin equivalent tissue culture model following treatment with HEX [review in Parisi et al, 2017].

The aromatic diamidines series has been studied to determine any enzyme inhibition properties. Geratz et al. [Geratz et al, 1973] examined their ability to inhibit trypsin, pancreatic kallikrein and thrombin; HEX dihydrate was effective against all enzymes with reported Ki values of 1.9, 4.5 and 7.4 µM, respectively.

Enyedy et al. [Enyedy et al, 2001] confirmed hexamidine inhibitory activity against thrombin although a considerably lower Ki value (224 nM) was reported; however, these authors did not specify if the active was used as the free base or salt form.

Furthermore, the study demonstrated that hexamidine was able to inhibit matriptase (Ki = 924 nM), a trypsin-like serine protease involved in tissue remodelling, cancer invasion and metastasis.

Finally, an *in vivo* study investigated the effect of two hexamidine salts on nitric oxide synthase (NOS) and found that the diisethionate salt significantly decreased NOS activity whereas the tetrachloroplatinate (II) salt had no effect on NO generation [Morgant et al, 1998].

The hydrochloride salt of hexamidine was found to be active against pneumonia induced in a rat model by a yeast-like fungus as *Pneumocystis carinii* [Review in Parisi et al, 2017].

2.4.2.3 SAFETY PHARMACOLOGY

The potential for hexamidine to produce adverse pharmacologic effects was evaluated on the digestive function in a four-week oral toxicity study in rats (refer to Section 2.4.4.1.2). All treated rats showed a slight caecum enlargement, an effect attributed to the antimicrobial properties of HEX D. The clinical signs and the caecum enlargement were not considered to be of toxicological significance. The no-observed-adverse-effect level (NOAEL) in this study was 50 mg/kg/day [CIR, 2007].

2.4.2.4 PHARMACODYNAMIC DRUG INTERACTIONS

No drug interactions with hexamidine have been reported in the international literature.

2.4.3 PHARMACOKINETICS

2.4.3.1 Absorption and distribution

In a pharmacokinetic study, HEX D was administered to Sprague Dawley (SD) rats at 10 mg/kg bw via the intravenous route (n = 5 rats) and at 50 or 200 mg/kg bw via a single oral dose (n = 5 rats/dose) [Zavorskas and Tozer, 2004 cited in CIR, 2007].

In the intravenous study, five rats received 10 mg/kg HEX D in a volume of 2 ml/kg saline by an infusion pump into the femoral vein over a dosing period of 15 min.

In the oral study, rats received a single dose of 50 or 200 mg/kg HEX D in 10 ml of 1% aqueous methylcellulose by oral gavage (n = 5 rats per dose level).

Blood was collected from each rat prior to dosing and at intervals up to 24 h after dosing. The blood samples were analyzed by liquid chromatography/mass spectrometry to determine the plasma concentration of HEX for each time point. The lower detection limit for this analytical method was 1.00 ng/ml.

After intravenous infusion of 10 mg/kg HEX D, the maximum plasma concentration (Cmax) of HEX was 2190 ng/ml; the mean residence time (MRT) was 5.0 h; the mean clearance was 10,700 ml/h/kg; the steady state volume of distribution was 389,000 ml/kg; and the half-life was 27.3 h.

After an oral dose of 50 mg/kg HEX D, the mean concentration-time profile was erratic, with plasma concentrations of Hexamidine decreasing after the first postdose sample collection and then increasing at the 2-h time point before declining again below the detection limit. In the rats receiving 200 mg/kg HEX D by oral gavage, the plasma concentration peaked after dosing, followed by a multiphasic decline measurable through 8 h post-dose.

In both oral dose levels, the time to Cmax was 15 min, indicating rapid absorption. The Cmax at 50 mg/kg was 3.10 ng/ml, and the Cmax at 200 mg/kg was 14.8 ng/ml.

Plasma concentrations of HEX were measurable up to 2 h after the 50 mg/kg dose and up to 8 h at 200 mg/kg. Oral bioavailability of Hexamidine was 0.10% at 50 mg/kg and 0.17% at 200 mg/kg [Zavorskas and Tozer, 2004 cited in CIR, 2007].

In a percutaneous study, it was found that HEX D was poorly absorbed by the skin of live rats. When the compound was applied as a 0.1 % formulation in cold cream under an occlusive dressing for 96 hours, a mean of ca. 0.6 % was absorbed (maximum value 1.4 %) [CIR, 2007; SCCNFP, 2002].

2.4.3.2 Metabolism and Elimination

HEX D given to rats intravenously was rapidly metabolized to HEX. Excretion was primarily via the feces, with a small amount excreted in the urine [CIR, 2007].

Following dermal application (occluded) of 56 µg/cm^2 HEX D 0.1 % on the shaved backs of rats, ≤ 2 % of HEX was absorbed over 96 hours and it was detected in the gastrointestinal tract, liver and kidneys. Less than 1 % of the total applied amount was excreted in the urine and 0.29 % in the faeces [CIR, 2007; IMAP, 2016].

No pharmacokinetics data after ocular administration of hexamidine in animals has been found in the literature. However, due to the poorly absorption of HEX D after oral administration and dermal application, no systemic passage of HEX D in the proposed formulation T1680 is expected after ocular administration.

2.4.4 TOXICOLOGY

The safety profile of T1680 is supported by literature toxicological data relative to hexamidine and its salt hexamidine diisethionate.

2.4.4.1 GENERAL TOXICITY

2.4.4.1.1 Single-dose toxicity

Acute toxicological data of HEX has been compiled in the Registry of Toxic Effects of Chemical Substances [RTECS, 2020] and reported on table hereafter.

Table 2.4.4.1-1: Hexamidine – Acute toxicity - LD_{50} Value [RTECS, 2020]

[redacted]

Regarding acute oral toxicity of HEX D, the median LD_{50} was found to be 2500 mg/kg in CFLP strain mice. Deaths occurred within 42 h of dosing. Observations at the 4.0 g/kg dose level included lethargy and piloerection shortly after dosing and ataxia and body tremors the day after treatment. Hemorrhage of the liver was observed in the animals that died.

Acute oral LD_{50} values of HEX D were 710 to 2500 mg/kg in mice and 750 mg/kg in rats [CIR, 2007] and 500 mg/kg in rabbits [SCCNFP, 2002].

Intraperitoneal toxicity values of 17-51 mg/kg bw and 57 mg/kg bw were reported for mice and rats, respectively. Intravenous values were 17 mg/kg bw for mice and 8 for rabbits [SCCNFP, 2002].

A dermal LD_{50} of >4000 mg/kg bw was reported in CYF rats for HEX D [SCCNFP, 2002].

Dermal application of up to 9.4 mL/kg of a 0.1 % solution (calculated to be 9.4 mg/kg bw) in rabbits did not cause mortalities or other toxicity effects [CIR, 2007].

Based on the available data, HEX D has low to moderate acute oral toxicity.

2.4.4.1.2 Repeat-dose toxicity

Multiple dose toxicity data of HEX has been compiled in the Registry of Toxic Effects of Chemical Substances [RTECS, 2020] and lowest published toxic doses (TDLo) are reported on below table.

Table 2.4.4.1-2: Hexamidine – Subacute toxicity – TDLo [RTECS, 2020]

[redacted]

[redacted]

Details of two repeated dose oral toxicity studies have been reported in the literature [CIR, 2007].

The first one was a **four-week oral toxicity study** of HEX D conducted in Sprague-Dawley rats. The animals were given 0, 50, 100, or 200 mg/kg/day HEX D (suspended in 1% aqueous methylcellulose) by oral gavage for 28 days (n = 5 rats/sex/dose level). No treatment-related deaths occurred. Body weights and food consumption were not affected by treatments. Salivation was the primary observation noted in all dose groups, with a slightly reduced incidence in the 50 mg/kg/day group. Associated wetness around the mouth with isolated incidences of brown oral staining began during the latter part of week 2 of dosing and continued to study termination. Males of the 200 mg/kg/day group had elevated mean total white blood cell counts attributable to lymphocytes. Increased alanine aminotransferase and serum calcium occurred in male rats of the 100 and 200 mg/kg/day groups. Increased aspartate aminotransferase was reported in the highest dose only. At necropsy, organ weights were similar between treated and control groups. All treated rats showed a slight caecum enlargement, an effect attributed to the antimicrobial properties of HEX D. The clinical signs and the caecum enlargement were not considered to be of toxicological significance. The no-observed-adverse-effect level (NOAEL) in this study was 50 mg/kg/day [CIR, 2007].

The second one was a **12-weeks oral study** conducted by gavage administration of 0, 200, 400, or 800 mg/kg/day HEX D to male rats, 5 days per week (n = 20 rats/dose level). There was no mortality in the 0 or 200 mg/kg/day groups; mortality rates were 30% at 400 mg/kg/day and 100% at 800 mg/kg at 4 weeks after treatment. There was a delay in body weight increases that appeared to be dose dependent. A decrease in weight gain first appeared in the 200 mg/kg/day group at week 11, in the 400 mg/kg/day group at week 6, and in the 800 mg/kg/day group at week 4. Slight anemia was seen in animals at the two highest doses. Except for increased transaminase activity and reduced renal clearance of creatinine, liver and kidney functions were not affected/impaired. HEX D showed no effect in the tissues examined histologically in any dose group [CIR, 2007].

Based on the available data, HEX D is not considered to cause severe effects following repeated oral exposure.

2.4.4.1.3 Genotoxicity

HEX D has been tested in the Ames bacterial reverse mutagenicity assay using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 with and without S9 microsomal activation. The

concentrations evaluated were 50, 150, 500, 1500, and 5000 µg/plate. Toxicity occurred at ≥ 500 µg/plate, so a second assay was prepared using 5, 15, 50, 150, and 500 µg/plate. HEX D did not induce reverse mutations in these assays [SCCNFP, 2002; CIR, 2007].

The effect of HEX D on chromosomal aberrations in Chinese hamster ovary (CHO) cells was evaluated. CHO cells were incubated with the test material at concentrations of 42, 210, 350, and 420 µg/ml with S9 microsomal activation and 3.4, 17, 27.5, and 34 µg/ml without S9 activation. There were no chromosomal aberrations after treatment without activation. In the presence of S9, a slight increase in the incidence of chromosomal aberrations was seen at the lowest dose but not in the higher doses. This finding was not considered to be indicative of a clastogenic effect. The authors concluded that Hexamidine Diisethionate had no evidence of clastogenic activity in this assay [SCCNFP, 2002; CIR, 2007].

Hexamidine Diisethionate does not appear to have mutagenic and clastogenic potential in the conventional tests.

2.4.4.1.4 Carcinogenicity

No published data were available on the carcinogenicity of HEX or HEX D [SCCNFP, 2002; CIR, 2007; IMAP, 2016] and the CIR Expert Panel concluded that HEX and HEX D are unlikely to be carcinogenic based on the negative genotoxicity studies and no structural alerts [CIR, 2007; IMAP, 2016].

However, some tumorigenic data on HEX has been found in the Registry of Toxic Effects of Chemical Substances [RTECS, 2020] and lowest published toxic doses (TDLo) together with the toxic effects found in this database are reported on below table.

Table 2.4.4.1-3: Hexamidine – Tumorigenic data – TDLo [RTECS, 2020]

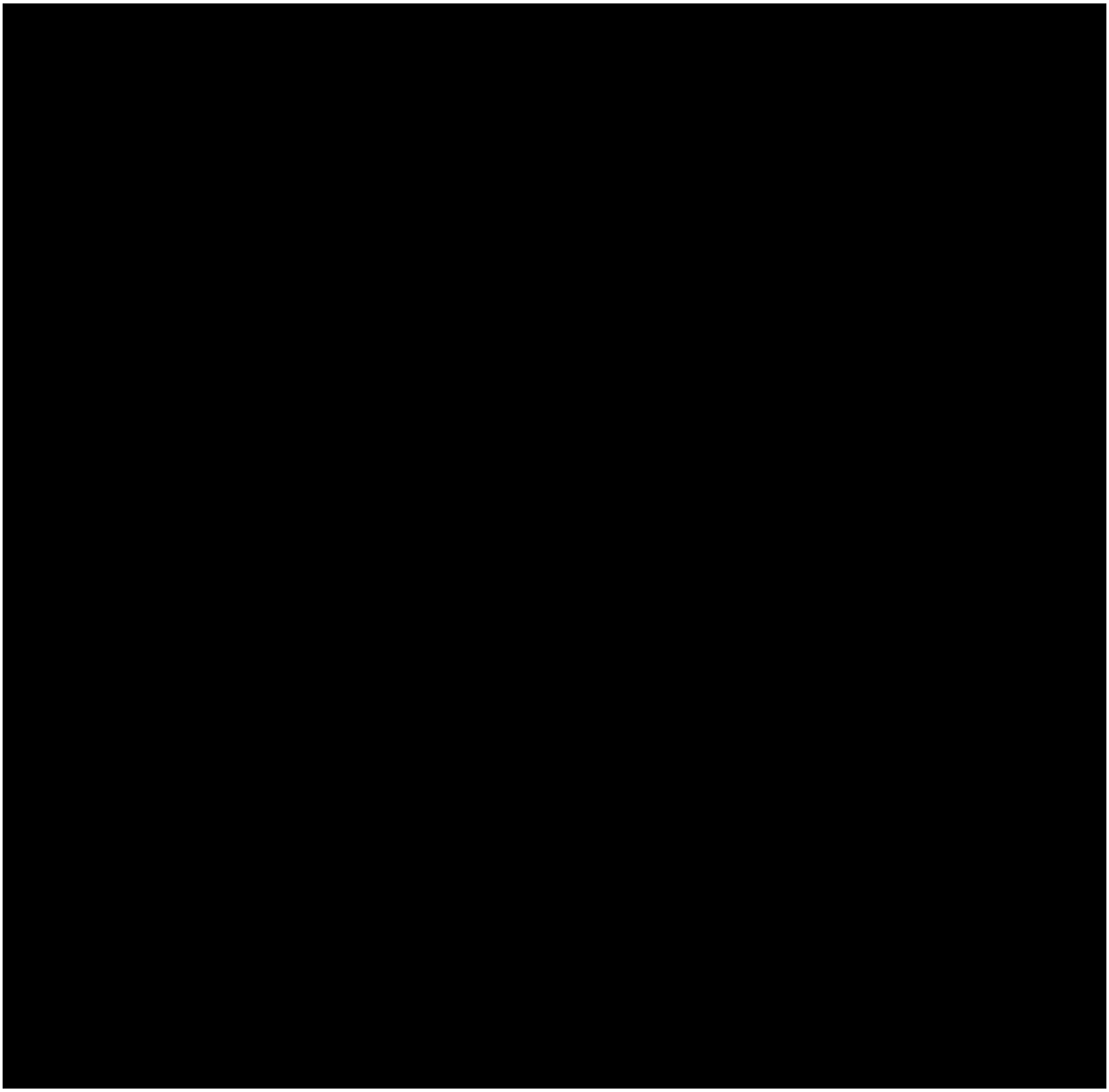
The limited timeframe (up to 8 days) for the clinical use of T1680 together with the negative genotoxicity studies and no structural alerts eliminates or at least reduces the need for structured carcinogenicity studies.

2.4.4.1.5 Reproductive and developmental toxicity

No published data were available on the reproductive or developmental toxicity of HEX or HEX D [SCCNFP, 2002; CIR, 2007; IMAP, 2016].

However, some reproductive data on HEX has been found in the Registry of Toxic Effects of Chemical Substances [RTECS, 2020]. The lowest published toxic doses (TDLo) together with the toxic effects found in this database are reported on below table.

Table 2.4.4.1-4: Reproductive data on hexamidine in animals [RTECS, 2020]



Because the proposed medicinal product is intended for ocular use at very low concentration (0.1%) for a short timeframe (up to 8 days) and because the rate of absorption of HEX D is low, with no tissue accumulation, rapid and complete excretion, it can be concluded that ocular exposures would not likely present a risk of reproductive/developmental toxicity.

Based on these considerations the topical administration of T1680 in the eye of lactating mothers reasonably should be considered as safe and harmless to the infant.

2.4.4.2 OCULAR TOXICITY

Two ocular toxicity studies have been performed in rabbits and reported in the literature [SCCNFP, 2002; CIR, 2007; IMAP, 2016].

In the first eye irritation study, New Zealand White rabbits (n = 3) were treated with 0.1 mL of HEX at 0.2 % (left eye) or 0.5 % (right eye) in propylene glycol. Their eyes were not rinsed and observations were made at one, 24, 48 and 72 hours after exposure.

Conjunctival reactions that persisted for up to three days were observed in all animals at 0.2 % (slight) and at 0.5 % (slight to moderate). No corneal opacity was observed. Ocular reactions were limited to the conjunctiva [SCCNFP, 2002].

In the other eye irritation study, 0.1 mL of 0.05 or 0.10 % of HEX D in aqueous solution was instilled in the right eye of albino rabbits (n = 9 /dose level). For each dose level, three animals' eyes were rinsed with lukewarm water for exactly two seconds after application of the tested solution, three animals' eyes were rinsed after four seconds, and three animals' eyes were not rinsed. The left eye of each animal served as control. The eyes were scored for signs of irritation 24, 48, and 72 hours and 4 and 7 days after the treatment.

Compared with the untreated left eyes, there were no reactions in the 0.05% dose group. Slight reactions were observed in eyes not rinsed of 0.10% HEX D but this observation disappeared after 72 hours. No reactions were observed in eyes rinsed of 0.10% HEX D 2 or 4 seconds after instillation [CIR, 2007].

HEX and HEX D have been reported to be a slight irritant in the rabbit eye after direct instillation [IMAP, 2016].

2.4.4.3 OTHER TOXICITY STUDIES

Three skin irritation studies have been reported in the literature [SCCNFP, 2002; CIR, 2007; IMAP, 2016]. Two studies were performed in rabbits and one in CYF rats.

In a skin irritation study in New Zealand White rabbits (n = 3), HEX was applied (occluded) at 0.2 or 0.5 % to the left or right flanks of the animals, respectively, for four hours. Observations were made at one, 24, 48 and 72 hours after treatment. One rabbit treated with 0.2 % and two rabbits treated with 0.5 % showed very slight erythema at 24 hours. No other reactions were observed. HEX was stated to be not irritating to the skin of rabbits [SCCNFP, 2002].

In another skin irritation study in albino rabbits (n = 6/dose), 0.5 mL of 0.05 or 0.10 % of HEX D was applied (occluded) to the flanks of the animals for 24 hours, with observation up to 72 hours. The right flank was abraded and the left flank left untouched. In the abraded sites exposed to 0.10 %, 'Light erythema' was reported in one rabbit at 24 hours, but not at 72 hours. The primary irritation indices were reported as zero and 1/12 for the 0.05% and 0.10 % treatments, respectively [CIR, 2007].

In the skin irritation study conducted in CYF rats (n = 5/sex/dose), a vehicle control or 4 g/kg of 40 % of HEX D was applied (occluded) to shaved areas (10 % body area) for 24 hours, with observation up to 14 days. Slight skin irritation (erythema or oedema) was observed in treated animals on day one (three females and one male) and on day two (two males). Although these effects were reversible by day three, a recurrence was observed on day six in two females. Slight scabbing was observed in four rats [CIR, 2007].

Based on the available data, HEX and HEX D have been considered to be slightly irritating to the skin in animal studies [IMAP, 2016].

Skin Sensitization

Hexamidine did not produce any evidence of sensitisation in guinea pigs nor of photosensitization using a rabbit model [CIR, 2007].

2.4.4.4 SAFETY ASSESSMENT OF EXCIPIENTS

The other ingredients included in the T1680 formula, identical to Desomedine® and in the same concentrations, are the followings:

- Borax and Boric acid used as buffering agents,
- Sodium chloride used to adjust the tonicity of the solution,
- Water for injection, the aqueous vehicle in which the ingredients are dissolved or dispersed.

All excipients are listed in the current European Pharmacopoeia and are well-known ingredients used in pharmaceutical preparations.

Since boron may impair fertility and may be harmful to babies, a safety limit is therefore set to 1 mg per day in the EMA guideline on excipients EMA/CHMP/302620/2017 Rev. 1.

For the proposed medicinal product, the maximum daily dose of boron, from borax and boric acid used as buffer agents, will be as follows:

Maximum daily dose = Boron per drop x D = 0.0759 * 12 = 0.911 mg/day

Where:

Quantity of Boron per drop = 0.0759 mg/drop

D = Maximal daily dose = 12 drops/day (1 drop 6 times a day per affected eyes)

The total dose of boron in the formula is less than 1 mg per day consequently no particular information is requested on the labelling and package leaflet of the proposed product as per the EMA/CHMP/302620/2017 Rev. 1

There is no toxicological concerns relating to the excipients of the T1680 formulation.

2.4.4.5 SAFETY ASSESSMENT OF IMPURITIES

[redacted]

MODULE 2 : COMMON TECHNICAL DOCUMENT SUMMARIES

2.4 NONCLINICAL OVERVIEW (T1680, 1mg/ml, Eye drops, solution)

2.4.5 INTEGRATED OVERVIEW AND CONCLUSIONS

Hexamidine is a strong organic base and is an aromatic diamidine. It has been used for its biocidal actions in topical preparations since the 1950s. It is primarily used as the diisethionate salt. HEX D is a hydrosoluble cationic agent with antimicrobial activity against bacteria, fungi, yeasts, and free-living amebae.

Laboratoires Théa developed a medicinal product T1680, a sterile preservative-free solution for ophthalmic use containing 1 mg/mL hexamidine diisethionate (0.1%). T1680 contains the same active substance and excipients in the same concentrations as the currently authorised product, Desomedine® registered by Bausch & Lomb for the single unit dose (reference product). The therapeutic indications and posology recommended for T1680 are the same as Desomedine®.

T1680 is indicated:

- for the treatment of:
    - purulent bacterial conjunctivitis caused by susceptible microorganisms
    - keratoconjunctivitis
    - blepharitis
    - chronic tear duct infections
- as a preoperative antisepsis for the conjunctival sacs

The recommended dose is one drop into the conjunctival sac of the affected eye(s), 4 to 6 times daily. The total duration of treatment should not exceed 8 days to avoid the emergence of resistant strains.

The pharmacological and toxicological particulars of T1680 as presented in this overview allow for the reasonable assumption that this product is safe and effective for the proposed indications.

Staphylococcus aureus, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa* are common causes of eye infection. Actually, *Staphylococcus epidermidis* is the organism most commonly isolated from eyes with post-operative endophthalmitis. Furthermore, *Pseudomonas* is the most frequent etiologic agent of contact lens-associated microbial keratitis, being responsible for up to 2/3 of cases. Fungi, including *Candida*, account for more than 50% of all culture-proven keratitis cases in tropical and subtropical regions and more than 50% of all cases of endogenous endophthalmitis.

In a very recent study HEX D solution showed rapid *in vitro* antimicrobial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Candida* species but was poorly active against *Pseudomonas aeruginosa*. These results confirm that hexamidine diisethionate remains in 2020 an efficient antimicrobial agent for common eye infections.

The exact mechanism of action of diamidines, including HEX is unknown, but they have been shown to inhibit oxygen uptake and induce leakage of amino acids, as would be expected if they are considered as cationic surface-active agents.

Pharmacokinetic studies found that HEX D is poorly absorbed orally and dermally. After intravenous administration, HEX D rapidly hydrolysed to HEX, with an elimination half-life of 27.3 hours. Excretion was primarily via the feces, with a small amount excreted in the urine. Oral bioavailability of hexamidine was 0.10 and 0.17 % at the oral doses of 50 or 200 mg/kg bw, respectively. Cmax at 50 or 200 mg/kg bw doses were 3.10 ng/mL and 14.8 ng/mL, respectively, after 15 minutes.

No pharmacokinetics data after ocular administration of hexamidine in animals has been found in the literature. However, in view of the poorly absorption of HEX D after oral administration and dermal application, no systemic passage of HEX D is expected after ocular administration of the proposed formulation T1680.

The satisfactory safety profile of T1680 ophthalmic solution is supported by relevant data available in the literature on the toxicology of HEX D.

Based on the available data, HEX D has low to moderate acute oral toxicity. No signs of toxicity were observed with 2% HEX D in subchronic studies in rabbits. The no-observed-effect level (NOEL) for oral subchronic toxicity of HEX D in rats was 50 mg/kg/day. HEX D is not considered to cause severe effects following repeated oral exposure.

HEX D does not appear to have mutagenic and clastogenic potential in the conventional tests. Because genotoxicity studies were negative, and there were no structural alerts, it was unlikely that HEX D would be carcinogenic.

No reproductive and developmental toxicity studies are described in the literature. Because the proposed medicinal product is intended for ocular use at very low concentration (0.1%) for a short timeframe (up to 8 days) and because the rate of absorption of HEX D is low, with no tissue accumulation and rapid and complete excretion, it can be concluded that ocular exposures of HEX D would not likely present a risk of reproductive/developmental toxicity. Based on these considerations the topical administration of T1680 in the eye of lactating mothers reasonably should be considered as safe and harmless to the infant.

HEX D has been reported to be a slight irritant after direct instillation in the rabbit eye in one irritation study. However, the relevance of the animal data is questionable because hexamidine eye drops are commonly use in clinical practice in humans for more than 20 years without any particular safety concerns.

The available toxicological data together with the tolerance data in human (refer to Module 2.5) can be used as bridging data for the proposed formula which is strictly identical to the one of the reference product.

Based on the available literature data, HEX and HEX D have been considered to be slightly irritating to the skin in animal studies.

The toxicological profile of the proposed medicinal product T1680 is dominated by the low dose (0.1%) which is applied to the eye, the short duration on treatment and reassuring toxicological data. In addition, HEX D has been widely used in topical cosmetics and medicinal products and is considered as safe and well tolerated product in clinical practice.

Results of various toxicological studies of the systemic toxicity have been described and justify the conclusion that the toxicological profile of HEX D has been sufficiently characterized and that additional studies with T1680 are not considered as needed. This also refers to the local toxicity of the product.

2.4.6 REFERENCES

2.4.6.1 LITERATURE REFERENCES

Aimard L, Brasseur P, Favennec L, Perrine D, Watt L, Brasseur G. Amebic keratitis due to a mixed infection with Acanthamoeba and Hartmanella species. Clin. Infect. Dis. 1998; 26:187–188.

Bailly C, Perrine D, Lancelot JC, Saturnino C, Robba M, Waring MJ. Sequence-selective binding to DNA of bis(amidinophenoxy)alkanes related to propamidine and pentamidine. Biochem. J. 1997; 323:23–31.

Brasseur G, Favennec L, Perrine D, Chenu JP, Brasseur P. Successful treatment of Acanthamoeba keratitis by hexamidine. Cornea. 1994; 13:459-462.

Cosmetic Ingredient Review (CIR) Expert Panel. Final report on the safety assessment of hexamidine and hexamidine diisethionate. Int. J. Toxicol. 2007; 26(Suppl. 3):79–88.

Enyedy, I.J., Lee, S., Kuo, A.H., Dickson, R.B., Lin, C. and Wang, S. Structure-based approach for the discovery of bis-benzamidines as novel inhibitors of matriptase. J. Med. Chem. 2001; 44:1349–1355.

Geratz, J.D., Whitmore, A.C., Cheng, M.C.F. and Piantadosi, C. Diamidino-a, w-diphenoxyalkanes. Structure-activity relations for the inhibition of thrombin, pancreatic kallikrein, and trypsin. J. Med. Chem. 1973; 16:970–975.

Granel F, Lozniewski A, Barbaud A, Lion C, Dailloux M, Weber M, Schmutz J. Cutaneous infection caused by Tsukamurella paurometabolum. Clin. Infect. Dis. 1996; 23:839–840.

Grare M, Dibama HM, Lafosse S. et al. Cationic compounds with activity against multidrug-resistant bacteria: interest of a new compound compared with two older antiseptics, hexamidine and chlorhexidine. Clin. Microbiol. Infect. 2010; 16:432–438.

IMAP (Accelerated Assessment of Industrial Chemicals in Australia) Group Assessment Report. Hexamidine and its diisethionate salt: Human health tier II. Assessment. Last Update 05 February 2016. https://www.nicnas.gov.au/chemical-information/imap-assessments/imap-group-assessment-report?assessment_id=2010 accessed on 20 April 2020

McDonnell, G. and Russell, A.D. Antiseptics and disinfectants: activity, action, and resistance. Clin. Microbiol. Rev. 1999; 12:147–179.

Morgant, G., Viossat, B., Roch-Arveiller, M. et al. In vivo nitric oxide synthase inhibitors can be deprived of this activity: unexpected influence of the tetrachloroplatinate(II) counteranion. Crystal structures of bis(Smethyl-isothiouronium)-N, N0-bis(3-guanidinopropyl)piperazinium and hexamidinium tetrachloroplatinates(II) salts. Met.-Based Drugs. 1998; 5:127–137.

Murray, D.S., Schumacher, M.A. and Brennan, R.G. Crystal structures of QacR-diamidine complexes reveal additional multidrug binding modes and a novel mechanism of drug charge neutralization. J. Biol. Chem. 2004; 279:14365–14371.

Parisi N, Paz-Alvarez M, Matts PJ, Lever R, Hadgraft J, Lane ME. Topical delivery of hexamidine. Int J Pharm. 2016;506(1-2):332-339.

Parisi N, Matts P, Lever R, Hadgraft J, Lane M. Hexamidine salts – applications in skin health and personal care products. International Journal of Cosmetic Science. 2017; 39(4):361-365.

Perez-Santonja JJ, Kilvington S, Hughes R, Tufail A, Matheson M, Dart JKG. Persistently culture positive Acanthamoeba keratitis: in vivo resistance and in vitro sensitivity. Ophthalmology. 2003; 110:1593–1600.

Perrine D, Chenu JP, Georges P, Lancelot JC, Saturnino C, Robba M. Amoebicidal efficiencies of various diamidines against two strains of Acanthamoeba polyphaga. Antimicrob. Agents Chemother. 1995; 39:339–342.

Pinna A, Donadu MG, Usai D, Dore S, Boscia F, Zanetti S. In Vitro Antimicrobial Activity of a New Ophthalmic Solution Containing Hexamidine Diisethionate 0.05% (Keratosept). Cornea. 2020;39(11):1415-1418.

SCCNFP (scientific advisory body to the European Commission in matters of consumer protection with respect to cosmetics and non-food products intended for consumers). Evaluation and opinion on: Hexamidine and its salts, including di-isethionate and di(p-hydroxybenzoate. 2002. https://ec.europa.eu/health/archive/ph_risk/committees/sccp/documents/out161_en.pdf accessed on 01-Oct-2020.

RTECS® – Registry of Toxic Effects of Chemical Substances. Hexamidine (4,6(1H,5H)-Pyrimidinedione, 5-ethyldihydro-5-phenyl-). US Department of Health and Human Services National Institute for Occupational Safety and Last update December, 2019. RTECS Number UV9100000, assessed on 17 November 2020

Taravaud A, Loiseau PM, Pomel S. In vitro evaluation of antimicrobial agents on Acanthamoeba sp. and evidence of a natural resilience to amphotericin B. Int J Parasitol Drugs Drug Resist. 2017;7(3):328-336.

van Ketel WG. Allergic contact eczema by Hexomedine. Contact Dermat. 1975; 1:332.

Vasseneix C, Gargala G, François A, Hellot MF, Duclos C, Muraine M, Benichou J, Ballet JJ, Brasseur G, Favennec L. A keratitis rat model for evaluation of anti-Acanthamoeba polyphaga agents. Cornea. 2006;25(5):597-602.

2.4.6.2 INTERNAL DATA

Dusart G. - Hexamidine: comparative study of the antibacterial activity of two eye drops, 10/24/2000.

2.4.6.3 INTERNAL REPORT

Allan F. - Toxicological assessment of Hexamidine Impurity A, January 2021