Hexamidine and its diisethionate salt: Human health tier II assessment

05 February 2016

Chemicals in this assessment

Chemical Name in the Inventory	CAS Number
Ethanesulfonic acid, 2-hydroxy-, compound with 4,4'-[1,6-hexanediylbis(oxy)]bis[benzenecarboximidamide] (2:1)	659-40-5
Benzenecarboximidamide, 4,4'-[1,6-hexanediylbis(oxy)bis-	3811-75-4

Preface

This assessment was carried out by staff of the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) using the Inventory Multi-tiered Assessment and Prioritisation (IMAP) framework.

The IMAP framework addresses the human health and environmental impacts of previously unassessed industrial chemicals listed on the Australian Inventory of Chemical Substances (the Inventory).

The framework was developed with significant input from stakeholders and provides a more rapid, flexible and transparent approach for the assessment of chemicals listed on the Inventory.

Stage One of the implementation of this framework, which lasted four years from 1 July 2012, examined 3000 chemicals meeting characteristics identified by stakeholders as needing priority assessment. This included chemicals for which NICNAS already held exposure information, chemicals identified as a concern or for which regulatory action had been taken overseas, and chemicals detected in international studies analysing chemicals present in babies' umbilical cord blood.

Stage Two of IMAP began in July 2016. We are continuing to assess chemicals on the Inventory, including chemicals identified as a concern for which action has been taken overseas and chemicals that can be rapidly identified and assessed by using

Stage One information. We are also continuing to publish information for chemicals on the Inventory that pose a low risk to human health or the environment or both. This work provides efficiencies and enables us to identify higher risk chemicals requiring assessment.

The IMAP framework is a science and risk-based model designed to align the assessment effort with the human health and environmental impacts of chemicals. It has three tiers of assessment, with the assessment effort increasing with each tier. The Tier I assessment is a high throughput approach using tabulated electronic data. The Tier II assessment is an evaluation of risk on a substance-by-substance or chemical category-by-category basis. Tier III assessments are conducted to address specific concerns that could not be resolved during the Tier II assessment.

These assessments are carried out by staff employed by the Australian Government Department of Health and the Australian Government Department of the Environment and Energy. The human health and environment risk assessments are conducted and published separately, using information available at the time, and may be undertaken at different tiers.

This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

For more detail on this program please visit:**www.nicnas.gov.au**

Disclaimer

ACRONYMS & ABBREVIATIONS

Grouping Rationale

Hexamidine diisethionate (CAS No. 659-40-5) is an organic salt of hexamidine (CAS No. 3811-75-4). Both chemicals are listed as biocides or preservatives in cosmetics and personal care products. Hexamidine diisethionate metabolises to hexamidine following intravenous (i.v.) administration (see **Toxicokinetics**) and, therefore, both chemicals are anticipated to display a similar hazard profile.

Import, Manufacture and Use

Australian

No specific Australian use, import, or manufacturing information has been identified.

International

The following international uses have been identified through: Galleria Chemica; the European Commission Cosmetic Ingredients and Substances (CosIng) database; the United States (US) Personal Care Products Council International Nomenclature of Cosmetic Ingredients (INCI) Dictionary; and the Cosmetic Ingredients Review (CIR).

The chemicals have reported cosmetic uses as:

- cosmetic biocides (to help cleanse the skin or prevent odour)

- preservatives;
- antifoaming agents; and
- emollients.

The CIR noted that hexamidine (CAS No. 3811-75-4) is not in current use in cosmetics, whereas hexamidine diisethionate (CAS No. 659-40-5) is reported to be used at concentrations of 0.03–0.10 % in various cosmetic products (CIR, 2007).

The following non-industrial uses have been identified internationally:

- hexamidine has reported use in over the counter medicines for babies at 0.05 %; and
- both chemicals have reported use as antiseptics and disinfectants for the treatment of minor infections in humans and in veterinary practice.

Restrictions

Australian

No known restrictions have been identified.

International

The chemicals are listed on the following (Galleria Chemica):

- EU Cosmetics Regulation 1223/2009 Annex V—List of Preservatives Allowed in Cosmetic Products (maximum concentration in ready for use preparation is 0.1 %); and
- New Zealand Cosmetic Products Group Standard—Schedule 7: Preservatives Cosmetic Products May Contain with Restrictions: List of Preservatives Allowed (at a maximum authorised concentration of 0.1 %).

Existing Worker Health and Safety Controls

Hazard Classification

The chemicals are not listed on the Hazardous Substances Information System (HSIS) (Safe Work Australia).

Exposure Standards

Australian

No specific exposure standards are available.

International

No specific exposure standards are available.

Health Hazard Information

Data for hexamidine and hexamidine diisethionate are used interchangeably, where required, to derive the toxicity of the chemicals, since the salt is hydrolysed to the parent chemical under biological conditions.

Toxicokinetics

Toxicokinetic studies are available for hexamidine diisethionate (CAS No. 659-40-5) in rats and human tissue. This chemical is poorly absorbed orally and dermally.

In a pharmacokinetic study, hexamidine diisethionate was administered to Sprague Dawley (SD) rats at 10 mg/kg bw via the i.v. route (n = 5 rats) and at 50 or 200 mg/kg bw via a single oral dose (n = 5 rats/dose). Following i.v. administration, hexamidine diisethionate rapidly hydrolysed to hexamidine (CAS No. 3811-75-4), with an elimination half-life of 27.3 hours. The maximum plasma concentrations (C_{max}) at 50 or 200 mg/kg bw doses were 3.10 ng/mL and 14.8 ng/mL, respectively, after 15 minutes. Oral bioavailability of hexamidine was 0.10 and 0.17 % at the oral doses of 50 or 200 mg/kg bw, respectively (CIR, 2007).

Following dermal application (occluded) of 56 µg/cm^2 hexamidine diisethionate (0.1 % formulation) on the shaved backs of rats, ≤2 % of the chemical 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).

In an in vitro penetration study in human cadaver skin, 3 or 5 mg/cm^2 hexamidine (0.1 %) in a gel or 0.0023 µg/cm^2 hexamidine (0.1 or 0.3 %) in an oil-water skin care formulation was reported to be poorly absorbed. The chemical penetrated the skin samples at 0.02–0.03 % in 72 hours. Due to the low skin penetration, bioavailability of hexamidine from topical cosmetic formulations is expected to be minimal (CIR, 2007).

Acute Toxicity

Oral

Based on the available data, the chemicals have moderate acute oral toxicity, warranting hazard classification (see **Recommendation** section).

The reported oral median lethal dose (LD50) values for hexamidine (CAS No. 3811-75-4) are:

- 2500 mg/kg bw in mice; and
- 500 mg/kg bw in rabbits (SCCNFP, 2002; CIR, 2007).

The reported oral LD50 values for hexamidine diisethionate (CAS No. 659-40-5) are:

- 750 mg/kg bw in rats; and
- 710 mg/kg bw in mice (SCCNFP, 2002; CIR, 2007).

Observed sub-lethal effects included lethargy and piloerection (immediate effect), and ataxia and body tremors (one day after treatment). Liver haemorrhage was observed in the animals that died (CIR, 2007).

Dermal

Based on the available data for hexamidine diisethionate, the chemicals have low acute dermal toxicity.

A dermal LD50 of >4000 mg/kg bw was reported in CYF rats for hexamidine diisethionate. 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).

Inhalation

No data are available.

Corrosion / Irritation

Skin Irritation

Based on the available data, the chemicals are considered to be slightly irritating to the skin in animal studies at low concentrations.

In a skin irritation study in New Zealand White rabbits (n = 3), hexamidine 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. The chemical 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 hexamidine diisethionate 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 a skin irritation study in CYF rats (n = 5/sex/dose), a vehicle control or 4 g/kg of 40 % of hexamidine diisethionate 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).

Eye Irritation

Based on the available data, the chemicals are considered to be slight eye irritants at low concentrations in animal studies.

In an eye irritation study in albino rabbits (n = 9/dose), 0.1 mL of 0.05 or 0.10 % of hexamidine diisethionate was instilled in the right eye and left un-rinsed (n = 3), or rinsed with lukewarm water after two seconds (n = 3) or four seconds (n = 3). Animals were observed at one, two, three, four and seven days after treatment. Compared with the untreated left eyes, there were no reactions at the 0.05 % dose level and in the rinsed eye at the 0.10 % dose level. Slight reactions which disappeared after 72 hours were observed in eyes of rabbits exposed at 0.10 % but not rinsed (CIR, 2007).

In another eye irritation study, New Zealand White rabbits (n = 3) were treated with 0.1 mL of hexamidine 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 and no irritation scores are available. Hexamidine was reported to be a 'slight irritant in the rabbits eye after direct instillation' (SCCNFP, 2002).

Sensitisation

Skin Sensitisation

Based on the available data for hexamidine diisethionate in animal studies, the chemicals are not considered to be sensitisers or photosensitisers at concentrations up to 50 %. Some evidence of sensitisation is available in human case reports, but not at a 0.1 % concentration in a skin sensitisation study (see **Observation in Humans**).

In a skin sensitisation study (non-guideline), white male guinea pigs (n = 10/dose) were intracutaneously administered hexamidine diisethionate (0.50 mL) at a concentration of 0.05 or 0.10 % into shaved skin, followed by a series of injections of 0.10 mL/day at the same concentration for a total of 10 injections per animal. Observations were made 24 hours after each injection. After a two week non-treatment period, the animals were challenged with 0.05 mL of the chemical at the same concentrations as they had previously received, by injection. There were no signs of irritation or sensitisation in this study (CIR, 2007).

In a Magnusson-Kligman study, female albino Hartley/Dunkin guinea pigs (n = 10) were intradermally induced with 1 % hexamidine diisethionate in liquid paraffin. This was followed by topical exposure to 0.4 mL of 50 % hexamidine diisethionate in liquid paraffin for 48 hours, one week after the injections. After a 14-day non-treatment period, the animals were challenged with occlusive patches of 25 % hexamidine diisethionate in liquid paraffin for a 24 hour period. Observations were made at 24, 48 and 72 hours. The chemical did not cause any reactions following the challenge dose. The authors concluded that the chemical 'did not produce any evidence of delayed contact hypersensitivity' (CIR, 2007).

In a photosensitisation test, albino rabbits (n = 3/sex) were exposed to saturated cotton patches of 0.1 % hexamidine diisethionate for 24 hours and exposed to UV light (five minutes/day), for 10 days (details not available). No differences in reactions between the treated and control animals were observed. It was reported in this study that the chemical is not a photosensitiser (CIR, 2007).

Quantitative Structure Activity Relationship (QSAR) modelling using the Organisation for Economic Cooperation and Development (OECD) Toolbox (version 3.3) gave no alerts for protein binding. QSAR modelling using OASIS-TIMES (version 2.27.16) resulted in a negative prediction for skin sensitisation. However, this prediction was out of the applicability domain, indicating uncertainty about the reliability of the prediction.

Observation in humans

Hexamidine diisethionate was not irritating or sensitising in a patch test at a concentration of 0.1 %. Several cases of skin sensitisation for both chemicals are available. Positive results have been observed at concentrations ≥1 %, although the study authors have stated that adverse reactions (such as allergy) to these chemicals are rare.

In a patch test in human subjects (n = 100/sex) at 0.1 % hexamidine diisethionate was applied in an aqueous solution (0.5 mL) to the left hand or in a cold cream formulation (0.5 g) to the right hand, for 24-hour periods. Exposures were repeated 10 times. After a 14-day non-treatment period, challenge patches of 0.10 % hexamidine diisethionate in aqueous solution or cold cream were applied to the same sites for 24 hours. The sites were evaluated for oedema, erythema and eschar formation. No signs of irritation, inflammation or sensitisation were observed in any of the subjects (CIR, 2007).

Several cases of contact sensitisation were reported for hexamidine diisethionate. Eight allergy cases were reported in male and female patients (aged 25–81 years). An eight-year old girl treated for irritation on the groin with an ointment containing hexamidine diisethionate developed sensitivity to the chemical (CIR, 2007). A seven-year old boy (with atopic dermatitis) developed a systemic allergic reaction after using a topical antiseptic cream. Skin prick tests with the active ingredients in the cream gave positive results for hexamidine diisethionate (10 % suspension in saline), but not the other ingredients (Mullins, 2006).

One case of photosensitivity and one case of contact sensitisation have been reported for hexamidine, in subjects with pre-existing eczema conditions. A 19-year old male eczema patient developed topographical lesions when a solution of hexamidine (concentration not stated) was applied to the skin, then exposed to sun. Patch tests were negative without UV exposure. In a case of contact sensitisation, symptoms worsened for a 13-year old boy with atopic eczema when treated with "Imacort" cream. The boy was patch tested with each ingredient in the cream formulation and sensitisation was observed for several ingredients including hexamidine (1 % in petrolatum) (CIR, 2007).

Repeated Dose Toxicity

Oral

Based on the available data for hexamidine diisethionate, the chemicals are not considered to cause severe effects following repeated oral exposure.

In a repeated dose toxicity study, male rats (n = 20/dose) were administered (by gavage) hexamidine diisethionate at 0, 200, 400 or 800 mg/kg bw/day, five days/week for 12 weeks. At ≥400 mg/kg bw/day, increased mortality rates, increased liver weights and slight anaemia were observed. Decreased body weight gain was observed at ≥200 mg/kg bw/day. Increased transaminase activity and reduced renal clearance of creatinine were observed (dose not stated), but liver and kidney functions were not adversely affected (SCCNFP, 2002; CIR, 2007). A no observed adverse effect level (NOAEL) was not determined.

In another repeated dose toxicity study, SD rats (n = 5/sex/dose) were administered (by gavage) hexamidine diisethionate at 0, 50, 100 or 200 mg/kg bw/day for 28 days. Clinical symptoms observed in all animals (but at a reduced incidence at the lowest dose) included salivation, wet fur and brown oral staining. At 200 mg/kg bw/day, abnormal position and locomotion were reported and, in males only, there were increased mean total white blood cells (lymphocytes). Increased alanine aminotransferase and serum calcium were observed at ≥100 mg/kg bw/day in males. Slight caecal enlargement observed in all rats was attributed to the antimicrobial properties of the chemical. No treatment-related effects were observed in the lungs, heart, liver, and kidneys. The authors established a NOAEL of 50 mg/kg bw/day in rats (SCCNFP, 2002; CIR, 2007)

Dermal

Based on the limited available data, the chemicals are not considered to cause severe effects following repeated dermal exposure.

In a repeated dose dermal toxicity study, rabbits (n = 3/dose) were exposed (shaved trunks) to hexamidine diisethionate solutions of 0.05, 0.1, 0.2 or 0.4 % (calculated to be 2, 4, 8 or 16 mg/kg bw), once daily for 90 days. There were no mortalities and no signs of irritation. There were no prominent observations at necropsy or following microscopic examination of tissues or in haematological parameters. The no observed effect level (NOEL) was 0.4 % (8 mg/kg bw) (CIR, 2007).

In a 28-day dermal toxicity study in rabbits, it was reported that daily application of the chemical (parent or salt not specified) at 0.05–2 % solutions (calculated to be 2–80 mg/kg bw) resulted in slight irritation only (SCCNFP, 2002). No other details are available.

Inhalation

No data are available.

Genotoxicity

Based on the limited data available, the chemicals are not considered to be genotoxic.

Negative results were reported in bacterial reverse mutation assays with several strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) using hexamidine diisethionate at concentrations up to 500 µg/plate, with or without metabolic activation. This chemical was not clastogenic in Chinese hamster ovary (CHO) cells up to 420 µg/mL with metabolic activation, or up to 34 µg/mL without metabolic activation. Although a slight increase in chromosomal aberrations was observed at the lowest dose (42 µg/mL) only, this was within the historical control range (SCCNFP, 2002; CIR, 2007).

No in vivo genotoxicity studies are available for the chemicals.

Hexamidine was observed to bind selectively to nucleotide sequences with at least four A-T base pairs (CIR, 2007).

QSAR modelling using OASIS-TIMES (version 2.27.16) resulted in a negative in vitro prediction (Ames), and positive in vivo predictions (micronucleus, comet and liver clastogenicity) for genotoxicity for both chemicals. However, the chemical structures were out of the applicability domain in all of the in vivo models, indicating uncertainty about the reliability of these predictions.

Carcinogenicity

No data are available.

QSAR modelling using the OECD Toolbox (version 3.3) gave no structural alerts for carcinogenicity for either chemical.

The CIR Expert Panel concluded that these chemicals were unlikely to be carcinogenic based on the negative genotoxicity studies and no structural alerts (CIR, 2007).

Reproductive and Developmental Toxicity

No data are available.

Risk Characterisation

Critical Health Effects

The critical health effect for risk characterisation is acute toxicity from oral exposure.

Public Risk Characterisation

The general public could be exposed dermally when using cosmetic products containing the chemicals. However, based on current use information reported by the CIR (2007), the concentration in these products (≤0.1 %) is not considered to be sufficiently high to cause health effects. Therefore, the risk to public health is not considered to be unreasonable and further risk management is not considered necessary for public safety.

Occupational Risk Characterisation

During product formulation, exposure may occur, particularly where manual or open processes are used. These could include transfer and blending activities, quality control analysis, and cleaning and maintaining equipment. Worker exposure to the chemicals at lower concentrations could also occur while using formulated products containing the chemicals. The level and route of exposure will vary depending on the method of application and work practices employed.

Given the critical systemic acute health effect, the chemicals could pose an unreasonable risk to workers unless adequate control measures to minimise exposure are implemented. The chemicals should be appropriately classified and labelled to ensure that a person conducting a business or undertaking (PCBU) at a workplace (such as an employer) has adequate information to determine the appropriate controls.

The data available support hazard classification of the chemical (see **Recommendation** section).

NICNAS Recommendation

Assessment of these chemicals is considered to be sufficient, provided that the recommended amendment to the classification is adopted, and labelling and all other requirements are met under workplace health and safety and poisons legislation as adopted by the relevant state or territory.

Regulatory Control

Work Health and Safety

The chemicals are recommended for classification and labelling under the current approved criteria and adopted GHS as below. This assessment does not consider classification of physical and environmental hazards.

Hazard	Approved Criteria (HSIS)[a]	GHS Classification (HCIS)[b]
Acute Toxicity	Harmful if swallowed (Xn; R22)	Harmful if swallowed - Cat. 4 (H302)

[a] Approved Criteria for Classifying Hazardous Substances [NOHSC:1008(2004)].

[b] Globally Harmonized System of Classification and Labelling of Chemicals (GHS) United Nations, 2009. Third Edition.

[*] Existing Hazard Classification. No change recommended to this classification

Advice for industry

Control measures

Control measures to minimise the risk from oral exposure to the chemicals should be implemented in accordance with the hierarchy of controls. Approaches to minimise risk include substitution, isolation and engineering controls. Measures required to eliminate, or minimise risk arising from storing, handling and using a hazardous chemical depend on the physical form and the manner in which the chemicals are used. Examples of control measures that could minimise the risk include, but are not limited to:

- minimising manual processes and work tasks through automating processes;
- work procedures that minimise splashes and spills;
- regularly cleaning equipment and work areas; and
- using protective equipment that is designed, constructed, and operated to ensure that the worker does not come into contact with the chemicals.

Guidance on managing risks from hazardous chemicals are provided in the *Managing risks of hazardous chemicals in the workplace—Code of practice* available on the Safe Work Australia website.

Personal protective equipment should not solely be relied upon to control risk and should only be used when all other reasonably practicable control measures do not eliminate or sufficiently minimise risk. Guidance in selecting personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

Obligations under workplace health and safety legislation

Information in this report should be taken into account to help meet obligations under workplace health and safety legislation as adopted by the relevant state or territory. This includes, but is not limited to:

- ensuring that hazardous chemicals are correctly classified and labelled;
- ensuring that (material) safety data sheets ((M)SDS) containing accurate information about the hazards (relating to both health hazards and physicochemical (physical) hazards) of the chemicals are prepared; and
- managing risks arising from storing, handling and using a hazardous chemical.

Your work health and safety regulator should be contacted for information on the work health and safety laws in your jurisdiction.

Information on how to prepare an (M)SDS and how to label containers of hazardous chemicals are provided in relevant codes of practice such as the *Preparation of safety data sheets for hazardous chemicals—Code of practice* and *Labelling of workplace hazardous chemicals—Code of practice*, respectively. These codes of practice are available from the Safe Work Australia website.

A review of the physical hazards of these chemicals has not been undertaken as part of this assessment.

References

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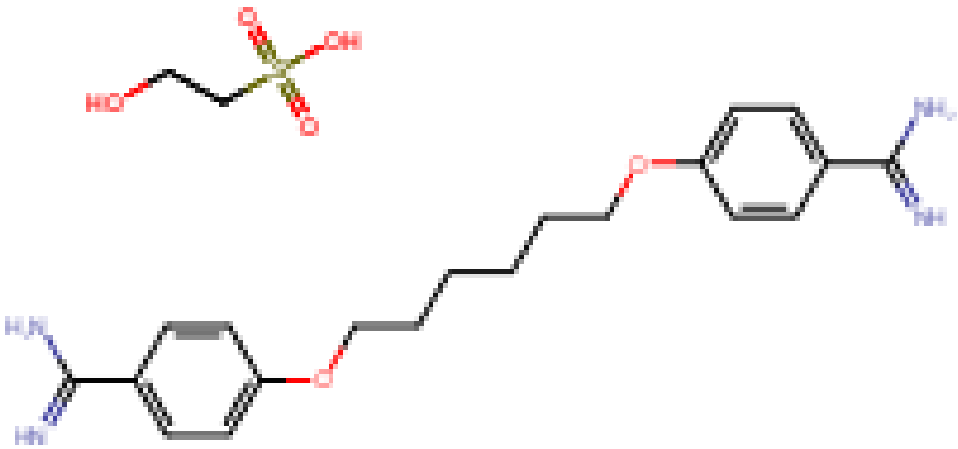
United States (US) Personal Care Products Council International Nomenclature of Cosmetic Ingredients (INCI) Dictionary. Accessed December 2015 at **http://gov.personalcarecouncil.org/jsp/gov/GovHomePage.jsp**

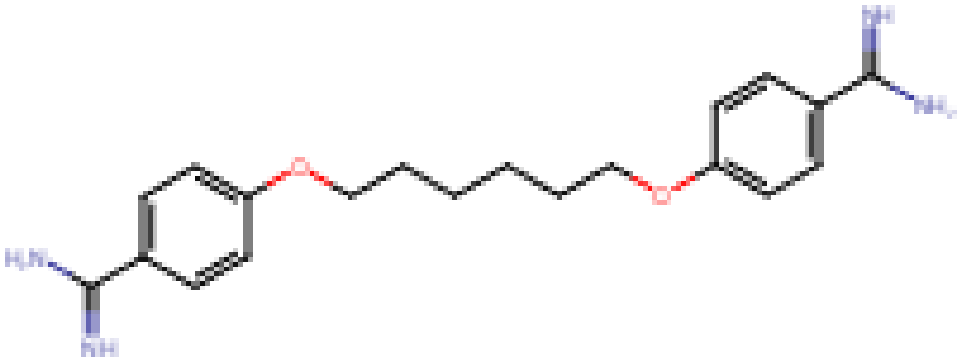
Last Update 05 February 2016

Chemical Identities

Chemical Name in the Inventory and Synonyms	**Ethanesulfonic acid, 2-hydroxy-, compound with 4,4'-[1,6-hexanediylbis(oxy)]bis[benzenecarboximidamide] (2:1)** hexamidine diisethionate benzenecarboximidamide, 4,4'-(1,6-hexanediylbis (oxy))bis-, diisethionate desmidine hexomedine benzamidine, 4,4'-(hexamethylenedioxy)di-, bis(2-hydroxyethanesulfonate)
CAS Number	659-40-5
Structural Formula	

	
Molecular Formula	C20H26N4O2.2C2H6O4S
Molecular Weight	606.71

Chemical Name in the Inventory and Synonyms	**Benzenecarboximidamide, 4,4'-[1,6-hexanediylbis(oxy)bis-** hexamidine benzamidine, 4,4'-(hexamethylenedioxy)di- 4,4'-(hexamethylenedioxy)dibenzamidine 4,4'-diamidino-1,6-diphenoxyhexane
CAS Number	3811-75-4
Structural Formula	

	
Molecular Formula	C20H26N4O2
Molecular Weight	354.45

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