PERMITTED DAILY EXPOSURE FOR SERRATIOPEPTIDASE

1. OBJECTIVE & SEARCH STRATEGY:

Determination of Health based exposure limits for a residual active substance through the derivation of a safe threshold value like Permitted daily exposure (PDE) or threshold of toxicological concern are used to determine the risk of the active pharmaceutical substance. For determination of PDE, all the available pharmacological and toxicological data including both non-clinical and clinical data should be evaluated. This involves hazard identification by reviewing all relevant data, identification of critical effects, determination of NOAEL of the findings that are considered to be critical effects.

In this document, brief summary of pharmacological, pharmacokinetics and toxicity data of Serratiopeptidase have been presented based on the published data. The data were extracted from PubMed, PubChem, TOXLINE, Drugdex, RTECS (Registry of Toxic effects of Chemical Substances), National Toxicology Program (NTP) and FDA.

2. INTRODUCTION:

Serratiopeptidase is a proteolytic enzyme (protease) produced by non-pathogenic enterobacterium Serratia sp. E-15, now known as Serratia marcescens ATCC 21074. This microorganism was originally isolated in the late 1960s from silkworm Bombyx mori L. (intestine), Serratiopeptidase is present in the silkworm intestine and allows the emerging moth to dissolve its cocoon. Serratiopeptase is produced by purification from culture of Serratia E-15 bacteria. It is a member of the Peptitase M10B (Matrixin) family.

3. IDENTITY OF THE ACTIVE SUBSTANCE:

Serratiopeptidase is prescribed in various specialities like surgery, orthopaedics, otorhinolaryngology, gynaecology and dentistry for its anti-inflammatory, anti-edemic and analgesic effects.

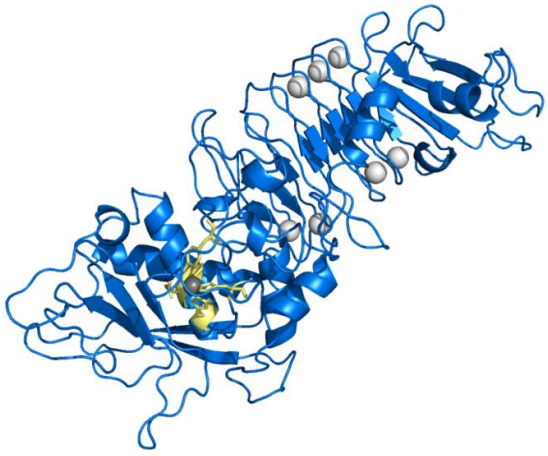
IUPAC name: Not Applicable

Chemical Abstract Services (CAS) Registry Number: 70851-98-8

Molecular Weight: Not Applicable

Chemical Formula: Not Applicable

Molecular Structure:



Crystal structure of serralysin with co-ordinated zinc (grey) and calcium (white).

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4. HAZARDS IDENTIFIED:

CATEGORIZATION:			
TOXICITY	**YES**	**NO**	**UNKNOWN**
Genotoxicant	-	√	-
Carcinogen	-	√	-
Reproductive/Developmental Toxicant	-	√	-
Highly Sensitizing potential	-	√	-

SUMMARY OF HAZARD IDENTIFICATION:	
Pharmacodynamics data	Serratiopeptidase is thought to work in three ways. **Anti-inflammatory:** Serratiopeptidase reduces swelling by the process of decreasing the amount of fluid in the tissues, thinning the fluid, and by facilitating the drainage of fluid. In addition, its enzyme activity dissolves dead tissue surrounding the injured area so that healing is accelerated. It may also act by modifying cell-surface adhesion molecules that guide inflammatory cells to their target site of inflammation. These adhesion molecules play an important role in the development of arthritis and other autoimmune diseases. **Analgesic:** It may help alleviate pain by inhibiting the release of pain-inducing amines like bradykinin from inflamed tissues. **Fibrinolytic/Caseinolytic:** It may be beneficial in atherosclerotic disease as it acts by breaking down fibrin and other dead or damaged tissue without harming living tissue. This could enable the dissolution of blood clots, and atherosclerotic plaques.
Pharmacokinetics data	After oral administration, Serratiopeptidase is absorbed through the intestines and transported directly into the bloodstream. But being a peptide there would be a high propensity of this enzyme to undergo enzymatic degradation in the gastrointestinal tract and low membrane permeability due to the hydrophilic nature of peptides and proteins in general. So these factors could lead to low bioavailability of this enzyme when used therapeutically. The intestinal absorption of Serratiopeptidase was assessed by measuring its concentration in plasma, lymph and extract of inflammatory tissue of rats by sandwich enzyme immunoassay (EIA) technique. Serratiopeptidase was administered orally to rats and was detected from plasma at 30 mg/kg dose and in lymph at 1 mg/kg. Its concentrations in plasma and lymph were dose dependent. It was seen that the peak concentration in plasma and lymph at a dose of 100 mg/kg were 0.87 ±0.41 and 43 ±42 ng/ml, respectively, and this peak plasma concentration was achieved 0.25-0.5 h after the dose and disappeared by 6 h. Serratiopeptidase was also detected in carrageenan-induced inflammatory tissue in animals at concentrations higher than that in plasma. It was concluded in the study that Serratiopeptidase is absorbed from the intestine, distributed to the inflammatory site via blood or lymph. Thus, indicating that orally administered Serratiopeptidase is absorbed from intestinal tract and reaches circulation in an enzymatically active form. In rat blood it exists as a complex with plasma protease inhibitor alpha-1 macroglobulin (a1M) with a molar binding ratio of 1:1 which helps to mask

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SUMMARY OF HAZARD IDENTIFICATION:

its antigenicity but still retains 20% of its original caseinolytic activity. However, pharmacokinetic data including its oral bioavailability in humans is not mentioned anywhere nor is the specific concentration required for its therapeutic action.

Acute Toxicity

Organism	Test Type	Route	Reported Dose (mg/kg)	Reference
Mouse	LD_{50}	i.p.	15.2	Drugs in Japan Vol. 6, Pg. 415, 1982
Mouse	LD_{50}	i.v.	14.23	Drugs in Japan Vol. 6, Pg. 415, 1982
Mouse	LD_{50}	Oral	2500	Drugs in Japan Vol. 6, Pg. 415, 1982
Mouse	LD_{50}	Subcutaneous	44	Drugs in Japan Vol. 6, Pg. 415, 1982
Rat	LD_{50}	i.p.	34.62	Drugs in Japan Vol. 6, Pg. 415, 1982
Rat	LD_{50}	i.v.	6.95	Drugs in Japan Vol. 6, Pg. 415, 1982
Rat	LD_{50}	Oral	2500	Drugs in Japan Vol. 6, Pg. 415, 1982
Rat	LD_{50}	i.v.	89	Drugs in Japan Vol. 6, Pg. 415, 1982

The studies in animals are very few. These have only demonstrated the anti-inflammatory effects of Serratiopeptidase. No specific model has been used in any of these studies to screen for the analgesic and anti-atherosclerotic effects of this enzyme in particular. In 3 animal studies Serratiopeptidase was shown to increase the antimicrobial concentration at the site of infection. While in 2 studies using anti-inflammatory animal models it was shown that Serratiopeptidase demonstrated significant anti-inflammatory activity when compared to chymotrypsin, trypsin, aspirin and Diclofenac. In another study, where Serratiopeptidase was compared to active comparator seaprose. Both enzymes showed reduction in viscosity of sputum, but the duration of therapy was not mentioned.

Repeated Dose Toxicity (Chronic Toxicity)	No data available
Carcinogenicity	No data available
In vivo/In vitro Genotoxicity Studies	No data available
Reproductive/Developmental Toxicity	Pregnant women and breast-feeding mothers should avoid the use of Serratiopeptidase since its efficacy isn't evaluated properly. If one is suffering from a bleeding disorder, the use of this medicine isn't recommended because of a few studies that claim that Serratiopeptidase might interfere with the process of blood clotting. If any surgery is scheduled in the near future, this medicine's use isn't recommended for two weeks prior to the surgery.
Highly Sensitizing Potential	Sensitivity to skin is very rare.

IDENTIFICATION OF CRITICAL EFFECTS:

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Sensitive Indicator of an adverse effect seen in non-clinical toxicity data	No any adverse effect seen in non-clinical toxicity data.
Clinical therapeutic and adverse effects	**Clinical Therapeutic Dose:** The dose mentioned in drug monographs ranges from 10 mg to 60 mg per day. The enzyme activity is measured in units. Serratiopeptidase 10 mg is equal to 20,000 units of enzyme activity. **Adverse Effects:** Serrapeptase was well tolerated in short-term clinical trials, but long-term safety has not been evaluated. Rare, serious adverse effects reported with serrapeptase include eosinophilic pneumonitis, bullous pemphigoid, hemorrhage in a patient with Behcet disease, and possibly Stevens-Johnson syndrome.

NOAEL/LOAEL	0.2 mg/kg/day considered as NOAEL (Minimum Therapeutic Dose)

APPLICATION OF ADJUSTMENT FACTORS:		
F1: Extrapolation between species	1	For extrapolation from rats to humans.
F2: Inter Individual Variability	10	Used for differences between individuals in the human population.
F3: Duration of Toxicity (Repeat Dose Toxicity)	10	Short duration study in rodent (14 days).
F4: Severe Toxicity (1-10)	1	No any toxicity (Genotoxicity/Reproductive toxicity/ Carcinogenicity) observed
F5: NOAEL or LOAEL (10 if LOAEL)	5	NOAEL value is selected (Minimum daily dose is selected in mg/kg/day).
PK Correction	For PDE calculation no pharmacokinetic correction was carried out	

CALCULATION	
PDE Calculation	$\frac{\text{NOEL or NOAEL or LOAEL (mg/kg/day) x Body Weight (kg)}}{\text{F1 x F2 x F3 x F4 x F5}}$ $= \frac{\text{0.2 (NOAEL) x 50}}{\text{1 x 10 x 10 x 1 x 5}}$ = 0.02 mg/day

5. REFERENCES:

- https://en.wikipedia.org/wiki/Serratiopeptidase
- https://www.drugs.com/npp/serrapeptase.html
- http://e-lactancia.org/media/papers/SerratiopeptidasaEficcia-IntJouSurg2013.pdf
- https://chem.nlm.nih.gov/chemidplus/rn/37312-62-2