PERMISSIBLE DAILY EXPOSURE (PDE) DETERMINATION STRATEGY FOR CLOTRIMAZOLE CREAM

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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 **Clotrimazole Cream** 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:

Clotrimazole is an antifungal medication. It is used to treat vaginal yeast infections, oral thrush, diaper rash, tinea versicolor, and types of ringworm including athlete's foot and jock itch. It can be taken by mouth or applied as a cream to the skin or in the vagina.

3. IDENTITY OF THE ACTIVE SUBSTANCE:

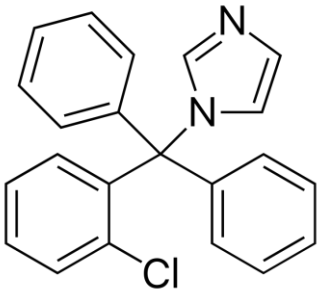
Clotrimazole is a white to pale yellow, crystalline, weakly alkaline substance, soluble in acetone, chloroform and ethanol, and practically insoluble in water. It forms stable salts with both inorganic and organic acids. It is not photosensitive but slightly hygroscopic and may be hydrolyzed in acid media.

IUPAC Name: 1-[(2-Chlorophenyl)(diphenyl)methyl]-1*H*-imidazole

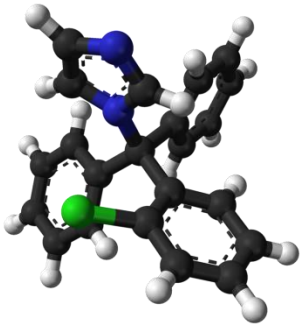
Chemical Formula: $C_{22}H_{17}ClN_2$

Molecular Weight: 344.84 g/mol

Molecular Structure:



2D



3D

4. HAZARDS IDENTIFIED:

CATEGORIZATION:			
Toxicity	**Yes**	**No**	**Unknown**
Genotoxicant	-	√	-
Carcinogen	-	√	-
Reproductive/Developmentaltoxicity	-	√	-
Highly sensitizingpotential	√	-	-

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5. SUMMARY OF ASSESSMENT PROCESS:

HAZARD IDENTIFICATION	
Pharmacodynamics data	Clotrimazole is a broad-spectrum antifungal agent that inhibits the growth of pathogenic yeasts by changing the permeability of cell membranes. The action of clotrimazole is fungistatic at concentrations of drug up to 20 mcg/mL and may be fungicidal *in vitro* against Candida albicans and other species of the genus Candida at higher concentrations Label. Unfortunately, resistance to clotrimazole, which was rare in the past, is now common in various patient populations. Clotrimazole is generally considered to be a fungistatic, and not a fungicidal drug, although this contrast is not absolute, as clotrimazole shows fungicidal properties at higher concentrations.
Pharmacokinetic data	Metabolism studies performed after oral or intravenous administration have shown that in most species studied, levels of Clotrimazole in tissue and serum are low. The majority of the drug is excreted as metabolites in the feces, with small amounts excreted in the urine. Human studies indicate slow excretion following oral administration of ^{14}C-labelled Clotrimazole (greater than 6 days). After intraperitoneal and subcutaneous administration, very low levels have been observed in the urine. Sitka reported levels of about 1% of the quantity of Clotrimazole in the 24-hour urine in newborns and premature infants. The absorption and organ distribution of the drug is very poor when administered parenterally. The pharmacokinetics of topically applied Clotrimazole in human subjects have been evaluated by Duhm et al. who reported on the penetration of radioactive Clotrimazole 1% cream and 1% solution into intact and acutely inflamed skin. Six hours after application of the drug, the concentration of Clotrimazole found in skin layers varied from 100 µg/cm3 in the stratum corneum to 0.5 to 1.0 µg/cm3 in the stratum reticulare and < 0.1 µg/cm^3 in the subcutis. No measurable amount of radioactivity (0.001 µg/mL) was found in the serum within 48 hours after application of 0.5 mL of the solution or 0.8 g of the cream. Sitka et al. reported serum levels of about 3 µg/mL in newborns and prematures and about 2.7 µg/mL in school children. Due to delayed excretion, prematures and newborns still showed values of 0.4 to 1.2 µg/mL after 24 hours; this level dropped faster to the zero point after 12 hours in older children.
Acute toxicity	Symptoms of overdose include erythema, stinging, blistering, peeling, edema, pruritus, urticaria, burning, and general irritation of the skin, and cramps. As with all topical agents, skin sensitization may result. **Oral LD_{50} (Rat)** : 708 mg/kg **Intraperitoneal LD_{50} (Rat)** : 445 mg/kg **Subcutaneous LDLO (Rat)** : 10 g/kg **Oral LD_{50} (Mouse)** : 761 mg/kg **Intraperitoneal LD_{50} (Mouse)** : 108 mg/kg **Acute dermal toxicity: LD50 (Mouse):** 923 mg/kg

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HAZARD IDENTIFICATION	
Repeated dose toxicity	**Species:** Rabbit **LOAEL:** 5 - 40 mg/kg **Application Route:** Skin contact **Exposure time:** 3 Weeks **Target Organs:** Skin **Symptoms:** Oedema, Fissuring, Necrosis, Redness **Species:** Rat **LOAEL:** 10 mg/kg **Application Route:** Oral **Exposure time:** 18 Months **Target Organs:** Liver, Kidney, Adrenal gland **Species:** Dog **LOAEL:** 25 mg/kg **Application Route:** Oral **Exposure time:** 6 - 12 Months **Target Organs:** Adrenal gland **Symptoms:** Salivation, Lachrymation, Vomiting.
Carcinogenicity	Carcinogenicity of Clotrimazole was evaluated in a 78-week oral dosing study in rats and the results did not show any carcinogenic effect of Clotrimazole.
Genotoxicity studies	Clotrimazole has been extensively studied in in vitro and in vivo mutagenicity assays, and no evidence of genotoxic potential was found. In an Ames test, an in vitro biological assay to detect the mutagenicity of chemical compounds, clotrimazole showed no evidence of mutagenic activity. Clotrimazole was found to be non-mutagenic in two additional in vitro studies, a gene mutation test in V79 cell lines and an Unscheduled DNA Synthesis (UDS) in primary rat hepatocytes. Studies evaluating the mutagenicity of clotrimazole in germ cells did not demonstrate mutagenic effects in a spermatogonia test in male hamsters, or in a dominant lethal test in male mice. Additionally, in mice, clotrimazole was not clastogenic in a micronucleus test.
Reproductive/Developmentaltoxicity	At dosages up to 100 mg/kg (oral), Clotrimazole was well tolerated by pregnant mice, rats and rabbits, and it had no embryotoxic or teratogenic effect. When given to pregnant rats at oral doses of 100 mg/kg from day 6 through day 15 of gestation, the number of resorptions was higher and the fetal weights were lower than the controls, but the number of fetal malformations did not differ significantly from that of the control group. Rats treated with Clotrimazole for 10 weeks at doses up to 50 mg/kg/day did not show any difference from the control group in the duration of estrus, fertility, duration of pregnancy, or in the number of implantations and

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HAZARD IDENTIFICATION	
	resorptions. The dose of 50 mg/kg/day impaired the development of the young, and dams receiving this dose level raised fewer offspring. **Effects on fertility:** Fertility/early embryonic development **Species:** Rat **Application Route:** Oral **Fertility:** LOAEL: 50 mg/kg body weight **Effects on fertility Effects on foetal development:** **Test Type:** Embryo-foetal development **Species:** Rat **Application Route:** Oral **Developmental Toxicity:** LOAEL: 100 mg/kg body weight **Result:** Embryo-foetal toxicity No teratogenic effects **Test Type:** Embryo-foetal development **Species:** Rat **Application Route:** Oral Developmental Toxicity: **NOAEL:** 50 mg/kg body weight **Result:** Embryo-foetal toxicity, No teratogenic effects **Test Type:** Embryo-foetal development **Species:** Mouse Application **Route:** Oral Developmental Toxicity: **NOAEL:** 200 mg/kg body weight **Result:** No effects on foetal development **Test Type:** Embryo-foetal development **Species:** Rabbit Application **Route:** Oral Developmental Toxicity: **NOAEL:** 180 mg/kg body weight **Result:** No effects on foetal development.
Highly sensitizing potential	**Primary skin irritation (patch test):** No detectable reddening on the intact rabbit skin at either 24 or 72 hours with Clotrimazole 1% solution or cream. Very slight erythema formation was observed after 24 hours in the scarified rabbit skin. **Primary irritation on conjunctival mucosa:** Clotrimazole solution or cream produced a transient conjunctival irritation in rabbits, consisting of low-grade reddening and a slight increase in secretion. No grossly detectable alterations were present in either the cornea or the iris of any of the treated animals. Both the cream and solution produced a transient, very slight reddening of the conjunctival mucosa. No alterations occurred on the cornea.

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HAZARD IDENTIFICATION	
Hazard Identification Symbol	 Irritant

IDENTIFICATION OF CRITICAL EFFECTS	
Clinical therapeutic and adverse effects	**Therapeutic Effect:** Vaginal clotrimazole is used to treat vaginal yeast infections in adults and children 12 years of age and older. Clotrimazole is in a class of antifungal medications called imidazoles. It works by stopping the growth of fungi that cause infection. **Adverse Effect:** When using Clotrimazole to treat vulvovaginal candidiasis, <10% of patients have a vulvar or vaginal burning sensation. Other side effects include rash, hives, blisters, burning, itching, peeling, redness, swelling, pain, or other signs of skin irritation.

NOAEL	**Species:** Rat; **LOAEL:** 10 mg/kg, **Application Route:** Oral, **Exposure time:** 18 Months

APPLICATION OF ADJUSTMENT FACTORS- PDE calculation		
F1: Extrapolation between species	5	Based on the selection of toxicity study of rats.
F2: Inter-individual variability	10	Conventionally used to allow for differences betweenindividuals in the human population
F3: Duration of toxicity	1	Long Term (18 Months)
F4: Severe toxicity (1-10)	1	No any toxicity
F5: NOAEL Vs LOAEL (10 if LOAEL)	10	Selection of LOAEL dose.

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PDE CALCULATION:

$$\text{PDE (mg/day)} = \frac{\text{NOAEL (mg/day) x Body weight (kg)}}{\text{F1x F2 x F3 x F4 x F5}}$$

$$= \frac{10 \text{ x } 50}{5 \text{ x } 10 \text{ x } 1 \text{ x } 1 \text{ x } 10}$$

= **1 mg/day**

6. REFERENCES:

- https://en.wikipedia.org/wiki/Clotrimazole
- https://pdf.hres.ca/dpd_pm/00031289.PDF
- https://go.drugbank.com/drugs/DB00257
- https://www.merck.com/docs/product/safety-data-sheets/ah-sds/Clotrimazole%20and%20Gentamicin%20and%20Betamethasone%20(0.1_pct)%20Formulation_AH_ID_6N.pdf

7. GLOSSARY:

PDE: Permitted daily exposure
AUC: Area under the curve **GRAS:**
Generally regarded as safe**GLP:** Good
laboratory practice
GMP: Good manufacturing practice
LD: Lethal dose
LED: Lowest-effective dose
TDLo (Toxic Dose Low): Lowest published toxic dose
LOAEL: Lowest-observed-adverse-effect level **LOEL:**
Lowest-observed-effect level
MSDS: Material safety data sheet
MTD: Maximum tolerable dose
MPDD: Maximum permissible daily dose
MTEL: Maximum tolerable exposure level**NEL:**
No-effect level
NOAEL: No-observed-adverse-effect level
NOEL: No-observed-effect level
OEL: Occupational exposure limit
QSAR: Quantitative structure–activity relationship

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SDS: Safety data sheet

ADI: Acceptable daily intake. Estimate by JECFA; the amount of a food additive, expressed on a body weight basis that can be ingested daily over a lifetime without appreciable health risk.

Area under the curve (AUC): Area between a curve and the abscissa (horizontal axis), i.e., the area underneath the graph of a function; often, the area under the tissue (plasma) concentration curve of a substance expressed as a function of time.

Bioaccumulation: progressive increase in the amount of a substance in an organism or partof an organism that occurs because the rate of intake exceeds the organism's ability to remove the substance from the body.

Bioavailability: biological and physiological availability. Extent of absorption of a substance by a living organism compared to a standard system.

Biological half-life: for a substance, the time required for the amount of that substance in a biological system to be reduced to one-half of its value by biological processes, when the rate of removal is approximately exponential.

Carcinogen: agent (chemical, physical, or biological) that is capable of increasing the incidence of malignant neoplasms, thus causing cancer.

Clastogen: agent causing chromosome breakage and (or) consequent gain, loss, or rearrangement of pieces of chromosomes.

Clearance: volume of blood or plasma or mass of an organ effectively cleared of a substance by elimination (metabolism and excretion) divided by time of elimination.

Cmax: used in pharmacokinetics referring to the maximum (or peak) serum concentrationthat a drug achieves in a specified compartment or test area after the drug has been administrated and before the administration of a second dose.

Critical dose: dose of a substance at and above which adverse functional changes, reversible or irreversible, occur in a cell or an organ.

Critical effect: for deterministic effects, the first adverse effect that appears when the threshold (critical) concentration or dose is reached in the critical organ:

Adverse effects with no defined threshold concentration are regarded as critical. Dose (of a substance): total amount of a substance administered to, taken up, or absorbed by an organism, organ, or tissue.

Draize test: evaluation of materials for their potential to cause dermal or ocular irritation and corrosion following local exposure; generally using the rabbit model (almost exclusively the New Zealand White) although other animal species have been used.

Elimination (in toxicology): disappearance of a substance from an organism or a partthereof, by processes of metabolism, secretion, or excretion.

Embryotoxicity: production by a substance of toxic effects in progeny in the first period of

pregnancy between conception and the fetal stage.

Fetotoxicity: production by a substance of toxic effects in progeny in the second period of pregnancy between fetal stage and delivery.

First-pass effect: biotransformation and, in some cases, elimination of a substance in theliver after absorption from the intestine and before it reaches the systemic circulation.

Gavage: administration of materials directly into the stomach by esophageal intubation.

Generally regarded as safe (GRAS): phrase used to describe the USFDA philosophy that justifies approval of food additives that may not meet the usual test criteria for safety but have been used extensively and have not demonstrated that they cause any harm to consumers.

Genotoxic: capable of causing a change to the structure of the genome.

Good laboratory practice (GLP) principles: fundamental rules incorporated in OECD guidelines and national regulations concerned with the process of effective organization and the conditions under which laboratory studies are properly planned, performed, monitored, recorded, and reported.

Hazard identification: determination of substances of concern, their adverse effects, target populations, and conditions of exposure, taking into account toxicity data and knowledge of effects on human health, other organisms, and their environment.

Hypersensitivity: state in which an individual reacts with allergic effects following exposure to a certain substance (allergen) after having been exposed previously to the same substance.

In silico: phrase applied to data generated and analyzed using computer modeling and information technology.

In vitro: in glass, referring to a study in the laboratory usually involving isolated organ, tissue, cell, or biochemical systems.

In vivo: In the living body, referring to a study performed on a living organism.

Lethal dose (LD): amount of a substance or physical agent (e.g., radiation) that causes death when taken into the body.

Lowest-effective dose (LED): lowest dose of a chemical inducing a specified effect in a specified fraction of exposed individuals.

Lowest published toxic dose (Toxic Dose Low, TDLo): the lowest dosage per unit of bodyweight (typically stated in milligrams per kilogram) of a substance known to have produced signs of toxicity in a particular animal species.

Lowest-observed-adverse-effect level (LOAEL): lowest concentration or amount of a substance (dose), found by experiment or observation, that causes an adverse effect onmorphology, functional capacity, growth, development, or life span of a target organism distinguishable from normal (control) organisms of the same species and strain under defined conditions of exposure.

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Lowest-observed-effect level (LOEL): lowest concentration or amount of a substance (dose), found by experiment or observation, that causes any alteration in morphology, functional capacity, growth, development, or life span of target organisms distinguishable from normal (control) organisms of the same species and strain under the same defined conditions of exposure.

Material safety data sheet (MSDS): compilation of information required under the U.S. OSHA Hazard Communication Standard on the identity of hazardous substances, health and physical hazards, exposure limits, and precautions.

Maximum permissible daily dose (MPDD): maximum daily dose of substance whosepenetration into a human body during a lifetime will not cause diseases or health hazards that can be detected by current investigation methods and will not adversely affect future generations.

Maximum tolerable dose (MTD): highest amount of a substance that, when introduced into the body, does not kill test animals (denoted by LD0).

Maximum tolerable exposure level (MTEL): maximum amount (dose) or concentration of a substance to which an organism can be exposed without leading to an adverse effect after prolonged exposure time. Maximum tolerated dose (MTD): high dose used in chronic toxicity testing that is expected on the basis of an adequate sub-chronic study to produce limited toxicity when

administered for the duration of the test period.

Median lethal dose (LD50): statistically derived median dose of a chemical or physical agent (radiation) expected to kill 50 % of organisms in a given population under a defined set of

conditions.

Mutagenicity: ability of a physical, chemical, or biological agent to induce (or generate) heritable changes (mutations) in the genotype in a cell as a consequence of alterations or loss of genes or chromosomes (or parts thereof).

No-effect level (NEL): maximum dose (of a substance) that produces no detectable changes under defined conditions of exposure.

No-observed-adverse-effect level (NOAEL): greatest concentration or amount of a substance, found by experiment or observation, which causes no detectable adverse alterationof morphology, functional capacity, growth, development, or life span of the target organism under defined conditions of exposure.

No-observed-effect level (NOEL): greatest concentration or amount of a substance, found by experiment or observation, that causes no alterations of morphology, functional capacity, growth, development, or life span of target organisms distinguishable from those observed in normal (control) organisms of the same species and strain under the same defined conditions of exposure.

Quantitative structure–activity relationship (QSAR): quantitative structure–biological activity model derived using regression analysis and containing as parameters physicochemical constants, indicator variables, or theoretically calculated values.

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Safety data sheet (SDS): single page giving toxicological and other safety advice, usually associated with a particular preparation, substance, or process.

Target (in biology): any organism, organ, tissue, cell or cell constituent that is subject to the action of an agent.