PERMITTED DAILY EXPOSURE FOR ALFACALCIDOL

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 Alfacalcidol 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:

Alfacalcidol (or 1-hydroxycholecalciferol) is an analogue of vitamin D used for supplementation in humans. Alfacalcidol has a weaker impact on calcium metabolism and parathyroid hormone levels than Calcitriol, however Alfacalcidol has significant effects on the immune system, including regulatory T cells. It is considered to be a more useful form of vitamin D supplementation, mostly due to much longer half-life and lower kidney load. It is the most commonly prescribed vitamin D metabolite for patients with end stage renal disease, given that impaired renal function alters the ability to carry out the second hydroxylation step required for the formation of the physiologically active form of vitamin D, 1,25-dihydroxyvitamin D_3. Alfacalcidol is an active vitamin D_3 metabolite and therefore does not require the second hydroxylation step in the kidney.

3. IDENTITY OF THE ACTIVE SUBSTANCE:

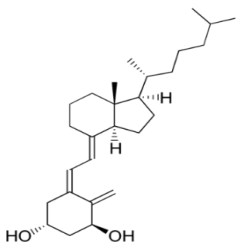
IUPAC NAME: (1R,3S,5Z)-5-[(2E)-2-[(1R,3aS,7aR)-7a-Methyl-1-[(2R)-6-methylheptan-2-yl]-2,3,3a,5,6,7-hexahydro-1H-inden-4-ylidene]ethylidene]-4-methylidenecyclohexane-1,3-diol

Chemical Abstract Services (CAS) Registry Number: 41294-56-8

Molecular Weight: 400.64 g/mol

Chemical Formula: $C_{27}H_{44}O_2$

Molecular Structure:



4. HAZARDS IDENTIFIED:

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

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SUMMARY OF HAZARD IDENTIFICATION:	
Pharmacodynamics data	Primary pharmacodynamics effects on bone Oral administration of Alfacalcidol in vitamin D–replete ovariectomised rats causes dose dependent suppression of osteoclastic bone resorption and stimulation of bone formation, resulting in a significant improvement in the quality as well as quantity of cortical and cancellous bone. In addition, Alfacalcidol is more effective in increasing cancellous bone mass in the skeletal sites with higher bone turnover. It was also shown that Alfacalcidol improved cancellous and cortical bone mass and bone strength by suppressing bone resorption and maintaining or even increasing bone formation. Bone strength depends not only on the quantity of bone tissue but also on its quality, which is characterised by the geometry and shape of bones, the microarchitecture of the trabecular bones, the mineral deposition, and the collagen quality. In rats, Alfacalcidol increases not only the amount of collagen but also enhances the maturation of collagen in ovariectomy-induced osteoporotic bones, which likely contributes to the improvement of bone quality. A combined treatment of Alfacalcidol increases muscle strength but does not affect muscle fatigue in ovariectomised rats. The effectiveness of activated vitamin D in preventing bone fractures may be partly owing to its effect on muscle strength in addition to its known effect on bone metabolism. **Secondary pharmacodynamics:** Osteoporosis Combination treatment of Alfacalcidol and risedronate at their sub therapeutic doses can improve the mechanical properties of the spine as well as the femur and ameliorate changes in calcium metabolism in a mouse model of osteoporosis. Furthermore, Alfacalcidol treatment increased cancellous and cortical bone mass and improved bone strength, resulting in the prevention of agerelated bone loss in aged male rats. Antitumor effect In mice inoculated with sarcoma cells, Alfacalcidol suppressed tumor growth or inhibited pulmonary metastases. Furthermore, Alfacalcidol has an systemic inhibitory effect on ornithine decarboxylase activity by tumour promotors.
Pharmacokinetic data	**Absorption and distribution:** In wild type and vitamin D deficient rats, intestinal absorption following oral administration of 24(S)-3Halfacalcidol was found to be 80% and 90%, respectively. The maximum plasma concentration of Alfacalcidol in rats was 4 hours. In dogs, a distribution half-life of 7 hours was observed following intravenous administration of 0.2 µg/kg bw of 3H-alfacalcidol, and the maximum plasma concentration of 1, 25-dihydroxyvitamin D3 was 0.218 pmol/ml at 4 to 6 hours after dosing. Following oral administration of 0.2 µg/kg bw of 3H Alfacalcidol, plasma levels of Alfacalcidol and 1,25-dihydroxy-vitamin D3 increased immediately with respective elimination half-lives (t1/2ß) of 5 and 8 hours and Cmax values of 0.265 and 0.328 pmol/ml at 4 hours after dosing. The Cmax of 1, 25-dihydroxy-vitamin D3 following oral dosing was higher and was achieved more rapidly than following intravenous dosing. This was attributed to significant firstpass metabolism following oral administration. Radioactivity following oral administration of 24(S)-3H-alfacalcidol was distributed mainly to plasma, liver and small intestinal mucosa in normal and vitamin D deficient rats. Distribution to the cytosol and nuclear fractions of small intestinal mucosa were also apparent. The amount of radioactivity recovered in faeces over a period of 6 days corresponds to 39% and 49% of the administered dose after intravenous and

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SUMMARY OF HAZARD IDENTIFICATION:

oral dosing, respectively. A small percentage of non-volatile metabolites was excreted in urine. It is known that metabolites that resulted from the metabolism of 1,25-dihydroxy-vitamin D3 are either less potent or biologically inactive.

Metabolism: One α-Hydroxyvitamin D3 (1(OH) D3) is a synthetic prohormone that can be converted to 1α, 25- dihydroxyvitamin D3 (1, 25(OH)2D3), the biologically active form of vitamin D3. This conversion occurs in the liver by the 25-hydroxylase activities of CYP3A4, CYP2J2, CYP27A1 and CYP2R1.

Acute Toxicity

Organism	Test type	Route	Dose (mg/kg)	Effect	Reference
Rat	LD_{50}	Oral	0.34	Sense organs and special senses: other changes: olfaction; endocrine: other changes; skin and appendages (skin): hair: other	Iyakuhin Kenkyu. Study of Medical Supplies., 8(560), 1977
Rat	LD_{50}	Subcutaneous	0.04	Null	PCT, #86-05395
Rat	LD_{50}	Intravenous	0.10	Sense organs and special senses: mydriasis (pupillary dilation): eye; sense organs and special senses: chromodacyrorrea: eye; blood: changes in spleen	Oyo Yakuri. Pharmacometrics., 15(653), 1978
Mouse	LD_{50}	Oral	0.44	Sense organs and special senses: lacrimation: eye; blood: changes in spleen; skin and appendages (skin): hair: other	Iyakuhin Kenkyu. Study of Medical Supplies., 8(560), 1977
Mouse	LD_{50}	Subcutaneous	0.055	Null	PCT, #86-05395
Mouse	LD_{50}	Intravenous	0.056	Lungs, thorax, or respiration: dyspnea; musculoskeletal: other changes; skin and appendages (skin): hair: other	Toxicology and Applied Pharmacology., 36(323), 1976 [PMID:1273850]
Dog	LD_{50}	Oral	0.5	Sense organs and special senses: lacrimation: eye; behavioral: altered sleep time (including change in righting reflex); gastrointestinal: hypermotility, diarrhea	Iyakuhin Kenkyu. Study of Medical Supplies., 9(103), 1978
Dog	LD_{50}	Intravenous	0.2	Sense organs and special senses: lacrimation: eye; behavioral: somnolence (general depressed activity)	Iyakuhin Kenkyu. Study of Medical Supplies., 9(103), 1978

Repeated Dose Toxicity (Chronic Toxicity)

Several oral repeated-dose toxicity studies (ranging from 1 to 12 months) were performed in Rats and Dogs.

Rat: **In a one month study in rat,** mortality, inhibited bodyweight gain, moderate leukocytosis, increased plasma calcium, total protein, total cholesterol, blood-urea nitrogen, and proteinuria occurred at doses of 12.5 µg/kg bw or higher. **The No Observed Adverse Effect Level (NOAEL) in this study was 0.5 µg/kg bw**.

In a three month study in rat, deaths occurred at 2.5 and 5 µg/kg bw. At doses of 0.1 µg/kg bw or higher effects observed included decreases of erythrocyte, leukocyte, lymphocytes, serum protein, albumin, glucose and potassium while neutrophils, serum and urinary calcium increased. Ectopic calcification typical of hypercalcaemia was present in kidney and heart. In a six month study in rats, bodyweight gain and food intake were decreased at 0.5 µg/kg bw/day and higher. An increase in serum and urinary calcium and inorganic phosphorus were present at doses of 0.1 µg/kg bw/day and higher. Histopathological studies revealed renal

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SUMMARY OF HAZARD IDENTIFICATION:	
	tubular degeneration, degeneration of cardiac muscle and blood vessel walls, atrophy of the medulla in the thymus gland and calcification of gastro-intestinal mucosa. **The NOAEL in this study was 0.02 µg/kg bw/day**. **Dog**: **In a one month study in dog,** at 1 µg/kg bw/day and higher, bodyweight, food intake, and lymphocytes were decreased, while urinary and plasma calcium concentrations, glutamicoxaloacetic transaminase, alkaline phosphatase, blood-urea nitrogen, total bilirubin and neutrophils were increased. Necropsy findings included hydrothorax, pulmonary oedema, cardiac muscle degeneration, white discoloration in kidney and gastro-intestinal tract, intestinal haemorrhage and atrophy of the thymus and reproductive organs. **The NOAEL was considered to be 0.04 µg/kg bw/day.** **In a one year dog study:** 0.08 µg/kg bw/day revealed reduced weight gain and food intake, ataxia, and emaciation, as well as a decrease in haematocrit, haemoglobin and erythrocyte counts. Increased serum calcium and inorganic phosphorus and blood-urea nitrogen were present. There were also effects on phenolsulphonphthalein excretion and glomerular filtration rate in the same dosage group. Histological changes (calcium deposition in the cavity and on the epithelial cells of renal tubules and atrophy of thymus cortex and medulla) were also observed in the 0.08 µg/kg bw/day group. **The NOAEL was 0.02 µg/kg bw/day.**
Carcinogenicity	The MAH did not provide a study or a statement on carcinogenicity.
In vivo/In vitro Genotoxicity Studies	The potential to induce reverse mutations in Salmonella typhimurium strains (TA98, TA100, TA1535, TA1538 and TA1537) was examined using the spot test and plate incorporation methods devised by Ames. Solutions in dimethyl-sulfoxide of 0.25 to 250 µg/plate were applied in the spot test and 250 µg/plate was found to represent the limit of solubility in the plate incorporation method. There were no increases in the numbers of revertant colonies, either in the presence or absence of metabolic activation. The potential of alfacalcidol to induce forward mutations at the thymidine kinase locus in cultured mouse lymphoma L5178Y cells in the absence and in the presence of metabolic activation was assessed. Alfacalcidol did not induce any dose-related or statistically significant increases in the frequency of mutant colonies.
Reproductive/Developmental Toxicity	Reproductive toxicity Embryo fetal toxicity studies were performed in rat and rabbit. In rat a reduction in maternal weight was observed at doses of 0.5 µg/kg bw/day and higher. At 2.5 µg/kg bw/day, increased intra-uterine death and fetal growth retardation including reduced ossification occurred secondary to severe maternal toxicity. In rabbit, maternal toxicity included diarrhoea, reduced feces and reduced body weight gain at all but the lowest dose tested (0.08-0.5 µg/kg bw/day). At all but the lowest dose fetal resorptions were increased and there was a dose dependent increase in number of abortions. **The fetal NOAEL in rabbits was 0.02 µg/kg bw/day.**
Highly Sensitizing Potential	Alfacalcidol occasionally causes skin rashes but other side-effects could be due to too much calcium being in your blood.

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IDENTIFICATION OF CRITICAL EFFECTS:	
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	Hypocalcaemia, Hypoparathyroidism, Hypophosphataemia, Renal Osteodystrophy, Rickets or Osteomalacia. **Adult:** Initially, 1 mcg daily. Maintenance: 0.25-1 mcg daily. **Child:** Premature infants and neonates: 0.05-0.1 mcg/kg daily; <20 kg: 0.05 mcg/kg daily. **Elderly:** 0.5 mcg daily.
NOAEL/LOAEL	The NOAEL was 0.02 µg/kg bw/day for Dog.

APPLICATION OF ADJUSTMENT FACTORS:		
F1: Extrapolation between species	2	For extrapolation from Dog 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 (1 year) study in non-rodent.
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
PK Correction	For PDE calculation no pharmacokinetic correction was carried out	

CALCULATION	
PDE Calculation	$\frac{\text{NOEL or NOAEL or LOAEL (mg/kg/day) x Body Weight (kg)}}{\text{F1 x F2 x F3 x F4 x F5}}$ $= \frac{0.02\ (\text{NOAEL}) \times 50}{2 \times 10 \times 10 \times 1 \times 5}$ = 0.001 mg/day

5. REFERENCES:

- https://pubchem.ncbi.nlm.nih.gov/compound/Alfacalcidol#section=Information-Sources.
- https://www.geneesmiddeleninformatiebank.nl/Pars/h119274.pdf.
- https://www.mims.com/malaysia/drug/info/alfacalcidol?mtype=generic.