PERMITTED DAILY EXPOSURE FOR VITAMIN B6

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 Vitamin B6 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:

Pyridoxine, also known as vitamin B_6, is a form of Vitamin B_6 found commonly in food and used as dietary supplement. As a supplement it is used to treat and prevent pyridoxine deficiency, sideroblastic anaemia, pyridoxine-dependent epilepsy, certain metabolic disorders, problems from isoniazid, and certain types of mushroom poisoning. It is used by mouth or by injection.

It is usually well tolerated. Occasionally side effects include headache, numbness, and sleepiness. Normal doses are safe during pregnancy and breastfeeding. Pyridoxine is in the vitamin B family of vitamins. It is required by the body to make amino acids, carbohydrates, and lipids. Sources in the diet include fruit, vegetables, and grain.

3. IDENTITY OF THE ACTIVE SUBSTANCE:

Pyridoxine is a water-soluble vitamin. Pyridoxine is composed of three forms (vitamers), pyridoxine, pyridoxal and pyridoxamine, all of which are normally present in foods. Pyridoxine hydrochloride is photosensitive and will degrade slowly when exposed to light.

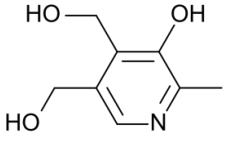
IUPAC name: 4, 5-Bis (hydroxymethyl)-2-methylpyridin-3-ol

Chemical Abstract Services (CAS) Registry Number: 65-23-6

Molecular Weight: 169.180 g·mol−1

Chemical Formula: $C_8H_{11}NO_3$

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	Vitamin B6 (pyridoxine) is a water-soluble vitamin used in the prophylaxis and treatment of vitamin B6 deficiency and peripheral neuropathy in those receiving isoniazid (isonicotinic acid hydrazide, INH). Vitamin B6 has been found to lower systolic and diastolic blood pressure in a small group of subjects with essential hypertension. Hypertension is another risk factor for atherosclerosis and coronary heart disease. Another study showed pyridoxine hydrochloride to inhibit ADP- or epinephrine-induced platelet aggregation and to lower total cholesterol levels and increase HDL-cholesterol levels, again in a small group of subjects. Vitamin B6, in the form of pyridoxal 5'-phosphate, was found to protect vascular endothelial cells in culture from injury by activated platelets. Endothelial injury and dysfunction are critical initiating events in the pathogenesis of atherosclerosis. Human studies have demonstrated that vitamin B6 deficiency affects cellular and humoral responses of the immune system. Vitamin B6 deficiency results in altered lymphocyte differentiation and maturation, reduced delayed-type hypersensitivity (DTH) responses, impaired antibody production, decreased lymphocyte proliferation and decreased interleukin (IL)-2 production, among other immunologic activities.
Pharmacokinetics data	**Absorption and bioavailability** The phosphate forms of Vitamin B6 in food are dephosphorylated in the intestinal lumen, and pyridoxine, pyridoxal and pyridoxamine are taken up from the small intestine by an energydependent process. All three are converted to pyridoxal phosphate in the tissues. A proportion of the vitamin B6 present in plant-based foods is biologically unavailable because it is present as pyridoxine glycosides that are not hydrolysed by intestinal enzymes. These glycosides may be absorbed, but do not act as a coenzyme in the body and are excreted unchanged in the urine. All three forms of vitamin B6 (pyridoxine, pyridoxal and pyridoxamine) are readily absorbed in the small intestine. The extent of absorption is decreased following gastric resection or in patients with malabsorption syndrome. Excess pyridoxine is excreted in the urine, and an adequate daily intake is therefore essential. **Distribution and metabolism** Pyridoxine in food is converted to active forms in the liver, a process which requires zinc and riboflavin. Vitamin B6 is stored in the liver, with about 50% also being present in muscle, bound to glycogen phosphorylase. Pyridoxine is also stored in the brain. The total body storage for adults is between 6 and 27 mg. Pyridoxine in the form of pyridoxal crosses the placenta, with foetal plasma concentrations being five times the level found in maternal plasma. The three forms of vitamin B6 are present in body tissues, mainly as 5-phosphorylated derivatives of pyridoxal and pyridoxamine. The half-life of pyridoxine is 15-20 days, and it is not significantly bound to plasma proteins. Pyridoxine, pyridoxal and pyridoxamine are all largely metabolised in the liver through phosphorylation by pyridoxal kinase. Pyridoxine phosphate is oxidised to the active coenzyme form, pyridoxal-5-phosphate, by an enzyme found mainly in liver. Pyridoxal-5-phosphate interconverts with pyridoxamine-5-phosphate through enzymatic transamination. The phosphorylated forms are hydrolysed by phosphatases. Pyridoxal is oxidised in the liver to pyridoxic acid. **Excretion** Pyridoxic acid, the main excretory metabolite, is eliminated via the urine.

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SUMMARY OF HAZARD IDENTIFICATION:

Acute Toxicity

Organism	Test type	Route	Dose (mg/kg)	Effect	Reference
Rat	LD50	Oral	4000	Behavioral: convulsions or effect on seizure threshold; behavioral: excitement	Arzneimittel-Forschung. Drug Research., 11(922) 1961 [PMID:14459216]
Mouse	LD50	Oral	5500	Behavioral: convulsions or effect on seizure threshold; lungs, thorax, or respiration: dyspnea	Arzneimittel-Forschung. Drug Research., 11(922) 1961 [PMID:14459216]
Mouse	LD50	Subcutaneous	2450	Behavioral: convulsions or effect on seizure threshold; lungs, thorax, or respiration: dyspnea	Arzneimittel-Forschung. Drug Research., 11(922) 1961 [PMID:14459216]
Mouse	LD50	Intravenous	660	Behavioral: convulsions or effect on seizure threshold; lungs, thorax, or respiration: dyspnea	Arzneimittel-Forschung. Drug Research., 11(922) 1961 [PMID:14459216]
Dog	LD50	Oral	500	Null	Arzneimittel-Forschung. Drug Research., 11(922) 1961 [PMID:14459216]
Cat	LD50	Oral	1000	Behavioral: convulsions or effect on seizure threshold; gastrointestinal: changes in structure or function of salivary glands; gastrointestinal: hypermotility, diarrhea	Arzneimittel-Forschung. Drug Research., 11(922) 1961 [PMID:14459216]
Cat	LD50	Intravenous	560	Behavioral: convulsions or effect on seizure threshold; gastrointestinal: changes in structure or function of salivary glands; gastrointestinal: hypermotility, diarrhea	Arzneimittel-Forschung. Drug Research., 11(922) 1961 [PMID:14459216]
Cat	LD50	Intramuscular	500	Behavioral: convulsions or effect on seizure threshold; gastrointestinal: changes in structure or function of salivary glands; gastrointestinal: hypermotility, diarrhea	Arzneimittel-Forschung. Drug Research., 11(922) 1961 [PMID:14459216]
Rabbit	LD50	Intravenous	464	Peripheral nerve and sensation: spastic paralysis with or without sensory change; behavioral: convulsions or effect on seizure threshold	Arzneimittel-Forschung. Drug Research., 11(922) 1961 [PMID:14459216]
Pigeon	LD50	Intravenous	145	Behavioral: altered sleep time (including change in righting reflex); behavioral: convulsions or effect on seizure threshold	Arzneimittel-Forschung. Drug Research., 11(922) 1961 [PMID:14459216]
Rat	LD50	Subcutaneous	3000	Behavioral: convulsions or effect on seizure threshold; behavioral: excitement	Arzneimittel-Forschung. Drug Research., 11(922) 1961 [PMID:14459216]
Rat	LD50	Intravenous	530	Behavioral: convulsions or effect on seizure threshold; behavioral: excitement	Arzneimittel-Forschung. Drug Research., 11(922) 1961 [PMID:14459216]

Repeated Dose Toxicity

Pyridoxine hydrochloride in Dogs:

• Males and females were treated orally (via gelatin capsules) with pyridoxine at increasing

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(Chronic Toxicity)	doses (50 mg/kg first week, 100 mg/kg second week, 150 mg/kg 3-16 weeks). • Anorexia and loss of body weight occurred in first weeks of trial, but stabilized to control levels at study end. •10/10 pyridoxine dogs developed neurologic disease (manifested by ataxia) including proprioceptive loss involving both fore- and hindquarters, characterized by stiff, spastic, dysmetric leg movements. Maintained muscle tone, but lacked apparent sense of motion or position of limbs. •Erythrocyte counts, hemoglobin concentration and packed cell volume were reduced, but considered "low normal". •Degenerative neurologic lesions limited to the dorsal funiculus, the trigeminal nerve fibers and the spinal tracts of the trigeminal nerves. Number of axons reduced and irregular/fragmented myelin. • The dose tested (150 mg/kg) is 121 times the highest proposed clinical dose of pyridoxine in Diclegis (40 mg).
Carcinogenicity	Two-year carcinogenicity studies in rats and mice were conducted at the U.S. National Center for Toxicological Research (NCTR). The rodents were administered doxylamine succinate at dose levels of 0, 500, 1000 and 2000 parts per million (ppm) in rats and dose levels of 0, 190, 375 and 750 ppm in mice. There were no increases in neoplastic lesions in female rats. Liver neoplasms in male rats were found only in the high-dose group. A trend test was significant ($p = 0.05$) for increased incidence of hepatocellular adenoma and carcinoma with increasing doses of doxylamine succinate, but the increased incidence of either lesion alone in the high dose group was not significant compared with controls. The incidence of these lesions was within the range historically observed in this strain of rats, and the results are not considered to have clinical relevance in humans. In the mouse bioassay, tumours that showed a statistically significant increase versus the control group in a trend test and in pairwise comparisons included hepatocellular adenomas and thyroid follicular cell adenomas. Doxylamine succinate produced a significant increase in hepatocellular adenomas in the mid to high dose group in male mice and the high dose group in female mice. There was no increase in the incidence of hepatocellular carcinomas in male mice and no hepatocellular carcinomas observed in any female mice. Thyroid follicular cell adenomas also were increased in treated mice of both sexes. These observations are consistent with a hormonal imbalance caused by induction of cytochrome P450 by doxylamine succinate in mice. Since enzyme induction is not observed in humans, doxylamine succinate is not considered to pose a carcinogenic risk under clinical use.
In vivo/In vitro Genotoxicity Studies	No data available
Reproductive/Developmental Toxicity	Tyl et al. studied a drug product containing equal concentrations of doxylamine succinate and pyridoxine hydrochloride in rats at doses of 0, 200, 500 and 800 mg/kg/day. Both maternal and fetal toxicity were evident at the two highest doses. Developmental toxicity included reduced prenatal viability and reduced fetal body weight per litter (500 and 800 mg/kg/day). No teratogenic effects of this drug were found even at the maternally toxic dose of 800 mg/kg/day. The finding of minor skeletal variations, such as a shortened 13th rib, only at the toxic high doses is consistent with general toxicity. Teratology studies in rabbits and reproduction studies in rats were conducted with doxylamine succinate alone, dicyclomine

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SUMMARY OF HAZARD IDENTIFICATION:	
	HCl alone, and a drug product containing a combination of doxylamine succinate, dicyclomine HCl and pyridoxine HCl. One of three groups of rats received 3-60 mg/kg/day of the combination, while the two other groups received 10-100 mg/kg/day of either dicyclomine or doxylamine. In the three rabbit groups, 3-30 mg/kg/day of the drug product containing the combination, and 10-100 mg/kg/day of either dicyclomine HCl or doxylamine succinate were given. No increase in congenital malformations or other adverse effects were noted in pregnancy when compared to nonexposed controls. None of these materials appeared to have any deleterious effects on reproductive parameters such as pregnancy maintenance, litter size, or fetal weight in the rabbit, except when toxic (100 mg/kg/day doxylamine succinate or dicyclomine HCl) levels were reached. In rats, these same drugs produced no alteration in breeding, conception, pregnancy maintenance, litter size, or fetal weight, although a mild dose-related decrease in neonatal weight gains occurred in pups from doxylamine succinate and dicyclomine hydrochloride-treated dams. In the first part of their investigation, Hendrickx et al. evaluated embryotoxicity of a combination of doxylamine succinate and pyridoxine hydrochloride in an uncontrolled small-scale study in preterm and term cynomolgus monkeys, rhesus monkeys and baboons. Some baboons received doxylamine succinate alone as opposed to the combination. Drugs were administered throughout the major period of organogenesis (gestation day 22 to 50). In these teratogenicity studies in the 3 species the treatment related effects of exposure to the combination of doxylamine succinate and pyridoxine hydrochloride in utero appear to be limited to a delay in closure of the ventricular septum that was evident at 100 days of gestation but not at term. Ventricular septal defects (VSD) were observed in 6 (40%) of the preterm cynomolgus monkeys, 2 (18%) of the preterm rhesus monkeys and 3 (23%) of the preterm baboons examined prenatally (day 100 of gestation). No dose response was evident and there were no other cardiac or extracardiac defects found except for one baboon fetus with multiple defects. No defects were observed in cynomolgus monkeys who were administered the combination of doxylamine succinate and pyridoxine hydrochloride for 4-day periods between 22 and 41 days of gestation. There was no association of this combination treatment with any noncardiac defect. In monkeys examined at term, there was no incidence of VSD, but one cynomolgous monkey had a mitral valve defect. This suggests an intrauterine delay in closure of the ventricular septum in monkeys, but that closure would occur before birth. The second part of this investigation examined the embryotoxic and teratogenic potential of doxylamine succinate and pyridoxine hydrochloride in term cynomolgus monkeys. The combination of doxylamine succinate and pyridoxine hydrochloride pulverized tablets or placebo were administered double-blind by nasogastric intubation on days 22-50 of gestation at doses approximately 2, 5 and 20 times the MRHD. Fetuses were delivered by caesarean section near term and examined. No congenital malformations were noted, and no evidence of embryo, fetal or maternal toxicity was observed.
Highly Sensitizing Potential	Few photosensitive reactions caused by Pyridoxine hydrochloride have been reported. There have been no reports of detailed photosensitivity studies on patients who have developed photosensitivity from pharmacologic doses of pyridoxine. Two patients with pyridoxine photosensitivity are described. Photo patch tests were positive for pyridoxine hydrochloride. The minimal erythematous dose (MED) for UVA decreased after the intravenous injection of pyridoxine hydrochloride in both patients. Normal controls gave negative results in photo patch tests for pyridoxine hydrochloride and photo test for vitamin B complex containing pyridoxine

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	hydrochloride. On the basis of these results, we believe both patients had a photo allergic reaction to pyridoxine hydrochloride.

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	**Clinical Therapeutic dose:** An adequate human diet in most circumstances is one containing between 1 and 2 mg vitamin B_6 daily. Doses of up to 150 mg daily have been used in general deficiency states. Doses of up to 100 mg daily from either the onset of symptoms or for 14 days prior to the start of menstruation have been used. **Adverse Effects:** • Clumsiness • numbness of hands or feet
NOAEL/LOAEL	3 mg/kg/day have been considered as NOAEL value (Maximum daily dose).

APPLICATION OF ADJUSTMENT FACTORS:		
F1: Extrapolation between species	1	No extrapolation done, maximum daily dose taken for evaluation.
F2: Inter Individual Variability	10	Used for differences between individuals in the human population.
F3: Duration of Toxicity (Repeat Dose Toxicity)	10	Data not available hence maximum value considered for factor.
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 (Maximum daily dose is selected in mg/kg/day).
PK Correction	For PDE calculation no pharmacokinetic correction was carried out	

CALCULATION	
PDE Calculation	$\frac{\text{NOEL or NOAEL or LOAEL (mg/kg/day) x Body Weight (kg)}}{\text{F1 x F2 x F3 x F4 x F5}}$ $= \frac{\text{3 (NOAEL) x 50}}{\text{1 x 10 x 10 x 1 x 5}}$ = 0.3 mg/day

5. REFERENCES:

- https://pdf.hres.ca/dpd_pm/00042353.PDF
- https://pubchem.ncbi.nlm.nih.gov/compound/Pyridoxine-hydrochloride#section=Acute-Effects
- https://www.ncbi.nlm.nih.gov/pubmed/9703142
- https://www.drugs.com/sfx/pyridoxine-side-effects.html