COLLEGE TER BEOORDELING VAN GENEESMIDDELEN

Public Assessment Report

Scientific discussion

Vitamine D Will 800 IU, 1000 IU and 3200 IU soft capsules (cholecalciferol)

NL/H/4946/003-005/DC

Date: 20 April 2026

This module reflects the scientific discussion for the approval of Vitamine D Will 800 IU, 1000 IU and 3200 IU soft capsules. The procedure was finalised on 30 August 2023. For information on changes after this date please refer to the 'steps taken after finalisation' at the end of this PAR.

List of abbreviations

25(OH)D	Calcifediol
1,25(OH)2D	Calcitriol
AE	Adverse Event
ASMF	Active Substance Master File
BHT	Butylhydroxytoluene
CEP	Certificate of Suitability to the monographs of the European Pharmacopoeia
CHMP	Committee for Medicinal Products for Human Use
CMD(h)	Coordination group for Mutual recognition and Decentralised procedure for human medicinal products
CMS	Concerned Member State
EAE	Experimental Autoimmune Encephalomyelitis
EDMF	European Drug Master File
EDQM	European Directorate for the Quality of Medicines
EEA	European Economic Area
EMA	European Medicines Agency
ERA	Environmental Risk Assessment
IBD	Inflammatory Bowel Disease
ICH	International Conference of Harmonisation
IU	International Unit
MAH	Marketing Authorisation Holder
MS	Multiple Sclerosis
Ph.Eur.	European Pharmacopoeia
PL	Package Leaflet
RA	Rheumatoid Arthritis
RH	Relative Humidity
RMP	Risk Management Plan
RMS	Reference Member State
SmPC	Summary of Product Characteristics
TSE	Transmissible Spongiform Encephalopathy
USP	United States Pharmacopeia
VDR	Vitamin D Receptor

I. INTRODUCTION

Based on the review of the quality, safety and efficacy data, the Member States have granted a marketing authorisation for Vitamine D Will 800 IU, 1000 IU and 3200 IU soft capsules, from Will-Pharma B.V.

The product (all strengths) is indicated for:

- Treatment of vitamin D deficiency (serum level 25(OH)D < 25 nmol/l or < 10 ng/ml) in adults and adolescents.

The product (800 IU and 1000 IU) is also indicated for:

- Prevention of vitamin D deficiency in adults with an identified risk.
- As an adjunct to specific therapy for osteoporosis in patients with vitamin D deficiency or at risk of vitamin D deficiency in adults.

A comprehensive description of the up-to-date indications and posology is given in the SmPC.

The marketing authorisation has been granted pursuant to Article 10a of Directive 2001/83/EC, which concerns a well-established use application.

This decentralised procedure concerns a bibliographical application based on well-established medicinal use of cholecalciferol and a line extension. This line extension is to introduce new dosages 800 IU, 1000 IU and 3200 IU to the already approved Vitamin D Will 25000 IU and 50000 IU soft capsules (NL/H/4946/001-002/DC) registered in the Netherlands by Will-Pharma B.V. since 10 August 2020.

For this type of marketing application (10a), the applicant needs to demonstrate that the active substance of the medicinal product has been in well-established medicinal use within the Community for at least 10 years in the specific therapeutic use. The results of non-clinical and clinical trials are replaced by detailed references to published scientific literature.

Cholecalciferol was first introduced into the European market at least ten years ago as a preoperative medication for treatment of vitamin D deficiency (serum level 25(OH)D < 25 nmol/l or < 10 ng/ml) in adults and adolescents, prevention of vitamin D deficiency in adults with an identified risk, and as an adjunct to specific therapy for osteoporosis in patients with vitamin D deficiency or at risk of vitamin D deficiency in adults.

The MAH submitted a justification for bridging between their already registered products (25000 IU and 50000 IU) and the product used in the literature. The bibliographic basis for the application of the lower strengths can be supported as the MAH justified that literature data is applicable to the Vitamine D Will 800 IU, 1000 IU and 3200 IU as well.

The concerned member states (CMS) involved in this procedure were Belgium and Luxembourg.

II. QUALITY ASPECTS

II.1 Introduction

Vitamine D Will 800 IU is a light yellow opaque, size 2, oval soft capsule. The capsule length is approximately 9.5 mm and width is approximately 5.9 mm. Each capsule contains as active substance 800 IU cholecalciferol, equivalent to 20 micrograms vitamin D_3.

Vitamine D Will 1000 IU is a orange opaque, size 2, oval soft capsule. The capsule length is approximately 9.5 mm and width is approximately 6.2 mm. Each capsule contains as active substance 1000 IU cholecalciferol, equivalent to 25 micrograms vitamin D_3.

Vitamine D Will 3200 IU yellow opaque, size 6, oval soft capsule. The capsule length is approximately 13.6 mm and width is approximately 8.4 mm. Each capsule contains as active substance 3200 IU cholecalciferol, equivalent to 80 micrograms vitamin D_3.

The excipients are:
Soft capsule fill - butylhydroxytoluene (BHT) and medium chain triglyceride oil.
Soft capsule shell - gelatine (E441), glycerol (E422), titanium dioxide (E171), iron oxide yellow (E172), iron oxide red (E172) (1000 IU only) and purified water.

The three tablet strengths are dose proportional.

The soft capsules are packed in white opaque polyvinyl chloride/polyvinylidene chloride/aluminium (PVC/PVDC/Alu) blisters.

II.2 Drug Substance

The active substance is cholecalciferol, an established active substance described in the European Pharmacopoeia (Ph.Eur.). The active substance is a crystalline powder and it is practically insoluble in water, freely soluble in ethanol (96%), and soluble in trimethylpentane and in fatty oils. Issues with regard to polymorphism are not relevant as the active substance is present in solution in the finished product.

The CEP procedure is used for the active substance. Under the official Certification Procedures of the EDQM of the Council of Europe, manufacturers or suppliers of substances for pharmaceutical use can apply for a certificate of suitability concerning the control of the chemical purity and microbiological quality of their substance according to the corresponding specific monograph, or the evaluation of reduction of Transmissible Spongiform Encephalopathy (TSE) risk, according to the general monograph, or both. This procedure is meant to ensure that the quality of substances is guaranteed and that these substances comply with the Ph.Eur.

Manufacturing process
A CEP has been submitted. Therefore no details on the manufacturing process have been included.

Quality control of drug substance
The active substance specification is considered adequate to control the quality and meets the requirements of the monograph in the Ph.Eur. and includes the additional test for a residual solvent stated on the CEP. In addition, a test for microbiological quality has been included in the drug substance specification of the drug product manufacturer. Batch analytical data demonstrating compliance with this specification have been provided for three batches.

Stability of drug substance
The CEP states a re-test period of 36 months when stored under the stated conditions. Assessment thereof was part of granting the CEP (and has been granted by the EDQM).

II.3 Medicinal Product

Pharmaceutical development
The product is an established pharmaceutical form and its development is adequately described in accordance with the relevant European guidelines. The choice of excipients is justified and their functions explained. The use and quantity of the antioxidant is adequately justified. The pharmaceutical development of the product has been adequately performed.

The intended use of the lowest strength in paediatric population has been adequately justified. As the active substance is already dissolved in the drug product, there is no reason to perform dissolution testing on the finished product. Thus, it is acceptable that no dissolution test was developed. In addition, as cholecalciferol is “practically insoluble in water”, there is no point in comparing dissolution profiles in physiological pHs, as required in the Bioequivalence Guideline, of the proposed formulation against other cholecalciferol products on the EU market or the products used in literature.

Manufacturing process
The manufacturing process consists of fill preparation, mixing and encapsulation. It has been validated according to relevant European guidelines. Process validation data on the product have been presented for one medium scale and two full scale fill material batches divided over three batches per strength, in accordance with the relevant European guidelines. Although the manufacturing process is considered a non-standard process due to low cholecalciferol content, the provided process validation data are considered sufficient in view of the long-term (30 years) experience of the drug product manufacturer with the manufacture of soft capsules.

Control of excipients
Reference to Ph. Eur. and USP is sufficient for the excipients. These specifications are acceptable.

Quality control of drug product

The finished product specifications are adequate to control the relevant parameters for the dosage form. The specification includes tests for appearance, identification cholecalciferol, average fill weight, average total weight, disintegration, loss on drying, uniformity of dosage units (mass variation, assay cholecalciferol, identification of BHT, assay of BHT, related substances, identification of colouring agents and microbiological examination. Limits in the specification have been justified and are considered appropriate for adequate quality control of the product. An adequate nitrosamines risk evaluation report has been provided. No risk for presence of nitrosamines in the drug product was identified.

Satisfactory validation data for the analytical methods have been provided.

Batch analytical data from three batches per strength from the proposed production site have been provided, demonstrating compliance with the specification.

Stability of drug product

Stability data on the product have been provided for at least three commercial scale batches per strength per supplier. A former and current active substance supplier were utilized for stability batch testing, as well as the use of a bracketing approach. The batches were stored at 25°C/ 60% RH (18 months), 30°C/75% RH (16 months) and 40°C/75% RH (6 months). The stability was tested in accordance with applicable European guidelines demonstrating the stability of the product for two years. Based on photostability results, the capsules should be stored in the original package in order to protect from light. On basis of the data submitted, a shelf life was granted of two years. The labelled storage conditions are “This medicinal product does not require any special temperature storage conditions. Store in the original package, in order to protect from light.”

Specific measures concerning the prevention of the transmission of animal spongiform encephalopathies

Scientific data and/or certificates of suitability issued by the EDQM cholecalciferol and gelatine have been provided and compliance with the Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via medicinal products has been satisfactorily demonstrated.

II.4 Discussion on chemical, pharmaceutical and biological aspects

Based on the submitted dossier, the member states consider that Vitamine Will D has a proven chemical-pharmaceutical quality. Sufficient controls have been laid down for the active substance and finished product.

No post-approval commitments were made.

III. NON-CLINICAL ASPECTS

III.1 Introduction

The MAH relied on the comprehensive scientific literature and collected a sufficient amount of information on pharmacological, pharmacokinetic and toxicological properties the active substance.

Based on the information reviewed and on the long experience in humans it seems that Cholecalciferol is essential to prevent and/or cure vitamin D deficiencies and associated risks. Vitamine D Will does not present any toxicological concern, since toxicity is only observed in exceeding doses far beyond those recommended for administration and therefore not relevant when the product is taken according to the directions laid out in the SmPC.

III.2 Pharmacology

Mode of action

Vitamin D is a fat-soluble vitamin that acts as a steroid hormone. The primary source of vitamin D is UVB induced conversion of 7-dehydrocholesterol to vitamin D in the skin. Vitamin D has a pivotal role as a calcaemic hormone, but it is now clear that vitamin D metabolites also have important non-calcaemic (non-classical) actions. The non-classical effects include actions on the cardiovascular system, regulation of innate and adaptive immune systems, a role in inflammatory and autoimmune diseases, release of insulin by pancreatic β cells and prevention of solid organ tumours (Dusso et al., 2005; Pilz et al., 2018; Nair and Maseeh, 2012).

Vitamin D undergoes two hydroxylations in the body for activation. The first one occurs in the liver and converts vitamin D to 25(OH)D, also known as calcifediol. The second one occurs primarily in the kidney and forms the physiologically active 1,25- dihydroxyvitamin D [1,25(OH)2D], also known as calcitriol. Calcitriol has a half – life of about 15 h while calcifediol (25(OH)D) has a half – life of about 15 days.

Vitamin D binds to vitamin D receptors (VDRs) throughout the body. 25(OH)D is transformed by renal or extrarenal 1α-hydroxylase into the active 1,25(OH)2D which circulates at much lower serum concentrations than 25(OH)D, but exerts a much higher affinity for the VDR. The enzyme of 1α-hydroxylase is also expressed in many other cell types including those of the vascular wall, and the conversion of 25(OH)D to the active 1,25(OH)2D happens at the level of the specific cell or tissue before being catabolised to the biologically inactive calcitroic acid. Moreover, there are many genes - modulated in part by Vitamin D – encoding proteins that regulate cell proliferation, differentiation, and apoptosis.

The active form of vitamin D – 1,25(OH)2D – acts through its specific zinc-finger nuclear receptor (VDR) analogous to the ones for oestrogens and retinoic acid. It enters the target cells to exert paracrine or endocrine effects, binds to the nuclear receptor VDR and induces a conformational change of the VDR that promotes its interaction with the retinoid X receptor

(RXR). The VDR/RXR complex induces transcriptional regulation of a variety of genes (Pilz et al., 2018; Nair and Maseeh, 2012; Christakos et al., 2016; Deluca, 2004).

Vitamin D promotes calcium absorption in the gut and maintains adequate serum calcium and phosphate to ensure normal mineralisation of bone and to prevent hypocalcaemic tetany. It is also needed for bone growth and bone remodelling by osteoblasts and osteoclasts. Vitamin D sufficiency prevents rickets in children and osteomalacia in adults. Together with calcium, vitamin D protects older individuals from osteoporosis.

Serum concentration of 25(OH)D is used as the best indicator of vitamin D status. It reflects vitamin D produced in the skin and that obtained from food and/or supplements and has a long circulating half-life of 15 days. However, serum 25(OH)D levels do not indicate the amount of vitamin D store in body tissues (Tolerable Upper intake levels for vitamins and minerals, Scientific Committee on Food Scientific Panel on Dietetic Products, Nutrition and Allergies, February 2006).

Vitamin D3 and autoimmune diseases - mice

The first experimental evidence of a link between vitamin D status and inflammatory bowel disease (IBD) comes from an animal model for IBD that was developed by Cantorna et al. Interleukin 10 (IL-10) knock-out mice that spontaneously develop symptoms resembling human IBD, were made to be vitamin D deficient or were supplemented with active vitamin D. Interestingly, treatment with 1,25(OH)2D3 treatment for as little as two weeks ameliorated IBD symptoms in these mice (Cantorna et al., 2000).

A mouse model for the human disease of multiple sclerosis (MS) has been developed in mice, the so-called experimental autoimmune encephalomyelitis (EAE) model. 1,25-dihydroxycholecalciferol [1,25-(OH)2D3] has been shown to inhibit the progression of EAE in mice while vitamin D deficiency resulted in an increased susceptibility of mice to EAE (Cantorna et al., 1996).

Rheumatoid arthritis (RA) is another autoimmune disease that can be imitated with two different animal models: murine Lyme arthritis and collagen – induced arthritis. Cantorna et al. also investigated the putative positive effects of vitamin D supplementation in mice that were infected with *Borrelia burgdorferi* (the causative agent of Lyme arthritis) or immunised with type II collagen. Supplementation with 1,25-(OH)2D3 minimised or prevented symptoms of arthritis in the treated group, while mice in the control group either developed severe arthritis or their symptoms worsened (Cantorna et al., 1996).

Vitamin D status and living environment - monkeys

In a recent small study in male Rhesus monkeys, Preston et al. showed that vitamin D status of those primates was highly depended upon sun exposure and dietary sources. The investigators assessed the vitamin D status in the blood of monkeys housed in high amounts of sunlight (corn-cribs), medium sunlight (corrals with shaded areas) and minimal sunlight (quarantine cages). 25 OH vitamin D which is the preferred metabolite to determine vitamin D status, was determined in the serum using High Performance Liquid Chromatography (HPLC). 25 OH vitamin D levels in blood were significantly greater in corn-crib housed animals than in coral or quarantine-housed animals ($p > 0.01$ and $p > 0.001$ respectively). Significant

differences of serum levels were not found when ages of animals housed in the same environment were compared. Those results emphasise the importance of the environment in which typically subjects spend their time when vitamin D results are interpreted (Preston et al., 2018).

Bone remodeling in hypervitaminosis D3 - rabbits

Normal bone growth and modelling is based on a balance between cartilaginous growth, maturation and resorption together with osteoblastic and osteoclastic activity. Both mechanisms require adequate blood supply. To investigate the mechanism of bone changes in vitamin D hypervitaminosis, experiments were designed in rabbits exposed to different doses of vitamin D, and radiographs were analysed at early stages and 6 to 12 weeks after vitamin D withdrawal. The rabbits of the control groups and those that received a small dose of Vitamin D3 (60000 IU per week for 1, 2 and 3 weeks) showed no change in radiography, microangiography or pathology. However, rabbits that received medium (300000 IU per dose for 3 doses with a 2-week interval between doses) or large doses of Vitamin D3 (3000000000 IU/kg/dose, 6 doses with 1-week interval between doses), showed morphologic changes with those being less severe in the medium dose group. Radiograms of the long bones and ribs showed subperiosteal resorption, linear intracortical lucencies, and periosteal new bone formation. The vascular ingrowth and the resorption of the calcified chondromatrix were abnormal. The metaphyseal and physeal changes are attributed to reinvasion of vessels between the calcified chondromatrix and physeal or articular cartilage, with recovery of normal endochondral ossification (Jiang et al., 1991).

III.3 Pharmacokinetics

Intestinal absorption and body retention of vitamin D was evaluated by Lorentzon and Danielson back in 1985. Tritiated Cholecalciferol ([3H]-D3) was intra-gastrically administered to rats previously fed with different amounts of vitamin D. From their results, animals with vitamin D deficiency accumulated high levels of serum radioactivity while they excreted less radioactivity in their 3-day faeces compared to animals without vitamin D deficiency (Lorentzon and Danielson, 1985).

Another study of Bikhazi and Hasbini investigated the brush-border mechanistic passage of vitamin D and 1,25(OH)2D metabolite. Radiolabelled cholecalciferol and 1,25(OH)2D were measured in intestinal perfusates and portal blood samples of rats injected with an inhibitor of protein and chylomicron synthesis. The amount of radiolabelled vitamin D lost from the perfusate was similar for the experimental and the control group of rats. However, treated rats showed a drastic increase in radiolabelled D3 retention in the intestine and a reduction in the portal plasma fraction (Silva and Furlanetto, 2018).

More recent *in vitro* studies with CaCo2 cells showed that long fatty acid chains that modulate cholesterol absorption also interfere with vitamin D absorption and that in mice cholecalciferol bioavailability was 15 times lower in mice in the presence of a phytosterol that is known to reduce dietary cholesterol absorption (Goncalves et al., 2013; Silva and Furlanetto, 2018).

From animal studies, *in vitro* studies and clinical studies in different groups of individuals, vitamin D bioavailability seems to be improved when vitamin D is given with fat containing food and is impaired by intestinal fat malabsorption (Silva and Furlanetto, 2018).

A very recent pre-clinical investigation aimed to obtain single dose pharmacokinetics in dogs from two different oral cholecalciferol formulations using corrective measures to overcome the interference of endogenous cholecalciferol. Thus, Patel et al. developed a fit for purpose method to ensure accurate and precise measurement of cholecalciferol to support the planned pharmacokinetic study comparing the two formulations of cholecalciferol in dogs. Even though numerous assays have been published that involve LC-MS/MS for the quantification of cholecalciferol in serum/plasma it is not easy to establish a method that would completely remove endogenous cholecalciferol and use a vitamin D3 – free serum environment for the comparative pharmacokinetic studies of two cholecalciferol formulations. In this preclinical study, 6 dogs were fasted overnight and received 60000 IU of cholecalciferol of reference and test product by mouth. Blood samples were collected on day 0 (baseline establishment) and after dosing on day 1 up to 28 days. The serum samples were extracted using protein precipitation/solid phase extraction and analysed to determine cholecalciferol by LC-MS/MS assay with calibrators prepared from cholecalciferol free serum. Standard pharmacokinetic analysis was carried out to assess pharmacokinetic parameters. Interestingly, serum cholecalciferol concentration vs. time profiles for the two formulations was almost superimposable. None of the PK parameters showed statistically significant differences ($p > 0.05$) between the two treatments. For example: C_{max} (ng/ mL) and AUC_{inf} (ng·h/mL) derived after the baseline corrections were 708.65 and 38 877.18 for reference and 743.71 and 40 665.51 for test, respectively. Pharmacokinetics of cholecalciferol was comparable between reference vs. test formulations. The procedures, baseline correction and employment of cholecalciferol devoid serum, can be readily adopted in future pharmacokinetic studies in animals or humans (Patel et al., 2017).

III.4 Toxicology

Toxic effects of vitamin D are related primarily to the role of free 1,25(OH)2D in plasma calcium regulation. Excessive production of the active vitamin D metabolite or greatly increased plasma 25(OH)2D may result in elevated plasma calcium levels due to over stimulated intestinal absorption and excessive calcium mobilisation from bone. Hypercalcaemia may also lead to an increased calcium excretion from the urine (hypercalciuria) (Vieth, 1990; Pettifor et al., 1995; Tolerable Upper intake levels for vitamins and minerals, Scientific Committee on Food Scientific Panel on Dietetic Products, Nutrition and Allergies, February 2006; Reichel et al., 1989). Hypercalcaemia is defined as a serum calcium above 2.75 mmol/L or ionised calcium above 1.35 mmol/L. Hypercalcaemia associated with hypervitaminosis leads to numerous debilitating effects such as loss of tubular concentration function of the kidney, reduced glomerular filtration rate, calcification of soft tissues etc. Several animal studies have been conducted involving systematic vitamin D intoxication over the past three decades in a variety of different species, including rats, cows, pigs, rabbits, dogs, and horses. As knowledge of vitamin D metabolism became more and more precise, focus of the research shifted to the levels of the metabolite 25(OH)D that must be exceeded to cause hypercalcaemia. Shephard and DeLuca proceeded to acute intoxication of rats with graded oral doses of Vitamin D3 (Jones., 2008).

Genotoxicology

Vitamin D3 was tested in *Salmonella typhimurium* assay at doses 0.033 to 10 mg/plate (strains TA1535, TA1537, TA97, TA98, TA100 were used) in the presence and presence of rat or hamster liver S9. Vitamin D3 was negative in this assay (Tolerable Upper intake levels for vitamins and minerals, Scientific Committee on Food Scientific Panel on Dietetic Products, Nutrition and Allergies, February 2006).

Acute toxicology – rats, dogs and rabbits

In early studies of 1975, the lethal dose of cholecalciferol in dogs was estimated to be 13 mg/kg of body weight. Immediate effects reported were bloody diarrhoea, anorexia, thirst, polyuria and prostration. In surviving animals, calcium was deposited as in chronic hypervitaminosis D (Tolerable Upper intake levels for vitamins and minerals, Scientific Committee on Food Scientific Panel on Dietetic Products, Nutrition and Allergies, February 2006). In 1980 Shephard and DeLuca proceeded to acute intoxication of rats with graded oral doses of Vitamin D3 (0.65 to 6500 ng/d for 14 d) or 25(OH)D3 (0.46 to 4600 ng/d for 14 d). They measured all major vitamin D metabolites by HPLC and competitive binding assays at the end of the dosing period. Vitamin D3 and 25(OH)D3 concentrations rose to micromolar levels in plasma of rats given the highest intakes of vitamin D3, resulting in marked hypercalcaemia. All dihydroxylated metabolites, including 24,25(OH)2D3; 25,26(OH)2D3; and 25(OH)D3-26,23-lactone, also rose to concentrations higher than 100 nmol/L, but the level of plasma 1α,25(OH)2D3 remained within the normal range. The study design did not allow for repeated measurements of plasma vitamin D metabolite levels as the hypercalcaemia developed in the animals, leaving open the possibility that plasma 1α,25(OH)2D3 initially rose and was subsequently suppressed in response to hypercalcaemia. Nevertheless, other animal studies of hypervitaminosis D have also repeatedly failed to demonstrate a significant elevation of the hormonal form. The 10-fold increments in the dose of vitamin D3 used in the study by Shephard and DeLuca did not allow for a very precise prediction of the 25(OH)D3 threshold that can be correlated with hypercalcaemia (toxicity), because a vitamin D3 dose of 65 ng/d resulted in a 25(OH)D3 concentration of 74±15 ng/mL (185±36 nmol/L) and no hypercalcaemia, whereas a vitamin D3 dose of 650 ng/d resulted in a 25(OH)D3 concentration of 643± 93 ng/mL (1608 ± 232 nmol/L) and profound hypercalcaemia (total calcium was 12.4 mg/dL). However, the studies with 25(OH)D3 revealed a remarkable finding: a dose of 460 ng/d resulted in 25(OH)D3 concentrations of 436 ± 53 ng/mL (1090 ± 132 nmol/L) with normocalcaemia. Although this study suggests that rodents can tolerate plasma 25(OH)D3 concentrations in the range of 250–1000 nmol/L, such tolerance represents a relatively unique situation for 25(OH)D3 administration, in which high circulating vitamin D3 that is present when vitamin D3 is the dietary form does not accompany elevated concentrations of 25(OH)D3. Based on a variety of studies in several animal species, it appears that the plasma 25(OH)D concentrations associated with toxicity are always in excess of 375 nmol/L. Furthermore, small differences between various mammalian species used in animal experiments are largely irrelevant to the toxicity issues (Jones et al., 2008).

Peixoto et al. investigated the effects of Vitamin D poisoning in rabbits by subcutaneous administration of an oily solution of cholecalciferol (non-activated Vitamin D3). The animals showed signs of cardiovascular insufficiency, as ascites and lung oedema, hyporexia, anorexia, mucous diarrhoea, loss of weight and apathy. The classical alterations of mineralisation and,

occasionally, ossification of the cardiovascular system, as well the lesions of kidneys, lungs, stomach, among other organs, were reproduced by the subcutaneous administration of an oily solution of cholecalciferol (non-activated vitamin D3) (Peixoto et al., 2010).

Chronic toxicity – rats/swine

Hypervitaminosis D in animals is associated with hypercalcaemia and adverse effects (AEs) secondary to that, as in humans. The severity of the symptoms and organ manifestations depend in the severity and length of the hypercalcaemia. Soft tissue calcifications were also common effects of hypervitaminosis D in animals. In a 26-week study focusing on the effects of vitamin D3 in the adrenal medulla of Charles River Crl:CD BR rats, animals were administered different daily doses of Vitamin D3 (0, 12, 25 and 50 µg/vitamin D3/kg of body weight) starting at 10 weeks of age. All different doses of vitamin D3 resulted in increased values of calcium and phosphorus levels and calcium excretion into the urine of rats. At 4 week the rats that were administered 12 and 25 µg of vitamin D3 / kg of body weight were presented with occasional foci of kidney tubular calcification and this was more prevalent in the high dose group of animals (50 µg/kg of body weight). At 26 weeks all kidneys from the high dose treated rats showed mild to moderate nephrocalcinosis, while rats treated with low to moderate doses showed either mild or no calcinosis, respectively. However, the effects of vitamin D3 in rats' adrenal medulla do not suggest a risk to humans. There is no evidence suggesting that hypercalcaemia is associated with pheochromocytomas (tumors of the adrenal glands) in humans and the rare occurrence of pheochromocytomas in patients with sarcoidosis is generally regarded as incidental (Tischler et al., 1999; Tolerable Upper intake levels for vitamins and minerals, Scientific Committee on Food Scientific Panel on Dietetic Products, Nutrition and Allergies, February 2006).

Toda et al., conducted a study to investigate the effects of different vitamin D3 dietary levels on the coronary arteries of 2-month old Yorkshire swine. Dietary vitamin D3 was given in doses of 2.5, 7.5, 50 and 100 µg vitamin D3/kg of body weight to different groups of swine for 4 months. The highest dose group was presented with thickening of the intima of the coronary vessels and increased levels of lipid containing – and degenerative cells. Those results are suggestive of a possible link between excessive vitamin D3 intake and the risk of development of human coronary atherosclerosis (Toda et al., 1985; Tolerable Upper intake levels for vitamins and minerals, Scientific Committee on Food Scientific Panel on Dietetic Products, Nutrition and Allergies, February 2006). However, Toda et al., used excessive doses of vitamin D3 to swine for up to 4 months and reported no significant changes in serum calcium and other markers (serum cholesterol, phospholipids etc.) which makes us doubt the validity of this study since other animal studies using comparable high doses of vitamin D3 to cattle or swine for example, reported increase in serum 25(OH)D accompanied by hypercalcaemia (Montgomery et al., 2000; Wilborn et al., 2004). Interestingly, hypercalcaemia induced by high oral doses of vitamin D resulted in nephrocalcinosis and coronary sclerosis in children (Zitterman, 2013).

<u>Reproductive toxicity - teratogenicity</u>

Vitamin D has been found to be teratogenic in animals when administered in doses 4 - 15 times the recommended human dose. Offspring from pregnant rabbits treated with high doses of vitamin D were presented with lesions reminiscent of those in cases of supravalvular aortic stenosis and others were presented with vasculotoxicity like the one that adults

experience upon acute vitamin D toxicity (Stockton and Paller, 1990; Tolerable Upper intake levels for vitamins and minerals, Scientific Committee on Food Scientific Panel on Dietetic Products, Nutrition and Allergies, February 2006).

Toda et al. also showed that 6-week-old piglets delivered from female pigs that received vitamin D3 highly enriched diets had more degenerated smooth muscle cells than those fed with low doses (Toda et al., 1985-b).

In a more recent study of 2012, Ogamba et al. investigated the effect of cholecalciferol over dosage on pregnancy outcome in white albino mice. They used 4 groups of pregnant female albino mice. In 3 groups they administered high doses of Vitamin D3 for a period of 22 days while the control group was only given saline and they studied parameters such as number of litters per delivery, average weight and length of the litters. The 3 experimental groups were treated with low dose 600 IU/kg, medium dose 1200 IU/kg or high dose 1800 IU/kg for 22 days. The number of litters was reduced only for the medium and the high dose treated group compared to the control group but there was significant reduction in the average weight and length of the litters of treated mice compared to the control ones. Overall, very high doses of Vitamin D negatively affected pregnancy outcome in white albino mice probably by inducing intrauterine growth retardation or down regulating the VDRs and inhibit fibroblast growth factor 23 (FGF-23) synthesis (Ogamba et al., 2011).

Vitamin D deficiency is common in pregnant women and is increasingly recognised as a public health problem. It is increasingly recognised that vitamin D has anti-inflammatory effects (Krishnan and Feldman, 2011). A 2011 report demonstrates that vitamin D regulates placental inflammation (Liu et al., 2011). Nevertheless, whether vitamin D protects against LPS-induced adverse developmental outcomes remain to be determined. A 2013 study in mice investigated the effects of supplementation with vitamin D3 during pregnancy on lipopolysaccharide (LPS)-induced neural tube defects (NTDs). Pregnant mice except controls were ip injected with LPS (25 µg/kg) daily from gestational day (GD)8 to GD12. In LPSþVitD3 group, pregnant mice were orally administered with VitD3 (25 µg/kg) before LPS injection. As expected, a 5-day LPS injection resulted in 62.5% (10/16) of dams and 20.3% of foetuses with NTDs. Additional experiment showed that a 5-day LPS injection downregulated placental proton-coupled folate transporter (pcft) and reduced folate carrier 1 (rfc1), 2 major folate transporters in placentas. Consistent with downregulation of placental folate transporters, folate transport from maternal circulation into embryos was disturbed in LPS-treated mice. Interestingly, supplementation with Vitamin D3 during pregnancy prevented LPS-induced NTDs through inhibiting placental inflammation and improving folate transport from maternal circulation into the embryos. Therefore, Vitamin D3 may have a potential preventive utility for protecting against LPS-induced developmental toxicity (Chen et al., 2015).

Studies on impurities

No studies on impurities were performed or provided.

Other toxicity studies - excipients

Butyl hydroxytoluene (BHT), is used as an antioxidant in cosmetics, foods, and pharmaceuticals. It is mainly used to delay or prevent the oxidative rancidity of fats and oils and to prevent loss of activity of oil-soluble vitamins. Butyl hydroxytoluene is also used at 0.5–1.0% w/w concentration in natural or synthetic rubber to provide enhanced color stability. Butylated hydroxytoluene has some antiviral activity and has been used therapeutically to treat herpes simplex labialis. Butylated hydroxytoluene is readily absorbed from the gastrointestinal tract and is metabolized and excreted in the urine mainly as glucuronide conjugates of oxidation products. Although there have been some isolated reports of adverse skin reactions, butylated hydroxytoluene is generally regarded as nonirritant and non-sensitizing at the levels employed as an antioxidant. The toxic effects of BHT are most commonly encountered in laboratory animals after chronic administration and refer to lesions in hepatic cells. Ingestion of 4 g of butylated hydroxytoluene, although causing severe nausea and vomiting, has been reported to be nonfatal (Rowe et al., 2009, 6th edition). Based on the various studies taken into consideration, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) defined an admissible daily intake (ADI) of 0-0.3 mg/kg body weight/day, which means 18 mg/day (Pifferi et al., 2003; JEFCA, Joint FAO/WHO Expert Committee on Food Additives, 51st Technical Report Series, 2000, p. 891).

BHT was evaluated for the oxidative protection of the final fill formulation of the proposed product. For the evaluation of the optimum BHT concentration the manufacturer performed two studies (measurement of peroxide value (Ip)_ and assay of vitamin D3, of BHT and related substances) to measure the effect on oils and on the final fill formulation. Based on the results of the studies, optimum concentration of BHT in the fill material was set to 0.10% (see 3.2.P.2 Pharmaceutical Development of the quality documentation). The release and shelf-life specification limit for the Assay of BHT for the proposed product was set to “90.0% to 110.0%” (see 3.2.P.5.1).

Medium-chain triglycerides (MCT) have been used in a variety of pharmaceutical formulations including oral, parenteral, and topical preparations. In oral formulations, medium-chain triglycerides are used as the base for the preparation of oral emulsions, microemulsions, self-emulsifying systems, solutions, or suspensions of drugs that are unstable or insoluble in aqueous media, e.g. calciferol. Medium chain triglycerides have also been investigated as intestinal absorption enhancers and have additionally been used as a filler in capsules and sugar-coated tablets, and as a lubricant or antiadhesion agent in tablets (Rowe et al., 2009, 6th edition).

Medium-chain triglycerides are generally regarded as essentially nontoxic and nonirritant materials. In acute toxicology studies in animals and humans, no irritant or other adverse reactions have been observed; for example, when they were patch-tested on more than 100 individuals, no irritation was produced on either healthy or eczematous skin. Medium-chain triglycerides are not irritating to the eyes. Similarly, chronic toxicology studies in animals have shown no harmful adverse effects associated with medium-chain triglycerides following inhalation or intraperitoneal, oral, and parenteral administration. In humans, administration of 0.5 g/kg body-weight medium chain triglycerides to healthy individuals produced no change in blood or serum triglycerides compared to subjects receiving the same dose of the long-chain triglyceride triolein. In patients consuming diets based on medium-chain triglycerides,

adverse effects reported include abdominal pain and diarrhea. Medium chain triglycerides are listed as generally recognized as safe (GRAS) and included in the FDA Inactive Ingredients Database (topical preparations). Included in non-parenteral and parenteral medicines licensed in Europe. Included in the Canadian List of Acceptable Non-medicinal Ingredients (Rowe et al., 2009, 6th edition).

There are already marketed medicinal products of vitamin D3 oily solutions in the EU that contain MCT such as, Benferol 800 IU, 5600IU, 25000 IU, 50000 IU, 100000 IU soft capsules/Consilient. Medium Chain triglycerides are considered physiochemically stable in comparison with other oils of vegetable origin such as arachis oil and maize oil that are often used in vitamin D3 formulations ("EMA/CHMP/302620/2017 corr. 1*). The proposed product "Vitamin D3 (Cholecalciferol) 800 IU, 1000 IU, 3200 IU, 20000 IU, 25000 IU, 50000 IU soft capsules" was formulated with MCT.

Gelatin is most frequently used to form either hard or soft gelatin capsules. Gelatin capsules are unit-dosage forms designed mainly for oral administration. Soft capsules are mainly filled with semi-solid or liquid fillings. Gelatin is soluble in warm water (>30^{o}C), and a gelatin capsule will initially swell and finally dissolve in gastric fluid to release its contents rapidly. The gelatin used to form the soft shells has a lower gel strength than that used for hard capsules, and the viscosity of the solutions is also lower, which results in more flexible shells. Additives to soft shell formulations are plasticizers here-in (glycerol 99.5%). Coloring and opacifying agents are also added. The filling can interact with the gelatin and the plasticizer chemically. There may be migration of filling components into the shell and plasticizer from the shell into the filler. These interactions must be considered during the formulation of the gelatin shell and the filling. In general, when used in oral formulations gelatin may be regarded as a nontoxic and nonirritant material. However, there have been rare reports of gelatin capsules adhering to the esophageal lining, which may cause local irritation. Hypersensitivity reactions, including serious anaphylactoid reactions, have been reported following the use of gelatin in parenteral products (Rowe et al., 2009, 6th edition). Gelatin is GRAS listed and included in the FDA Inactive Ingredients Database (dental preparations; inhalations; injections; oral capsules, pastilles, solutions, syrups and tablets; topical and vaginal preparations). It is also included in medicines licensed in the UK, Europe, and Japan and in the Canadian List of Acceptable Non-medicinal Ingredients (Rowe et al., 2009, 6th edition).

Titanium dioxide is widely used in foods and oral and topical pharmaceutical formulations. It is generally regarded as an essentially nonirritant and nontoxic excipient. It is widely used in confectionery, cosmetics, and foods, in the plastics industry, and in topical and oral pharmaceutical formulations as a white pigment. It is accepted as a food additive in Europe and included in the FDA Inactive Ingredients Database (dental paste; intrauterine suppositories; ophthalmic preparations; oral capsules, suspensions, tablets; topical and transdermal preparations). Included in non-parenteral medicines licensed in the UK. Included in the Canadian List of Acceptable non-medicinal Ingredients (Rowe et al., 2009, 6th edition).
Iron oxides are widely used in cosmetics, foods, and topical pharmaceutical applications as colorants and UV absorbers. However, iron oxides also have restrictions in some countries on the quantities that may be consumed, and technically their use is restricted because of their limited color range and their abrasiveness. They are generally regarded as nontoxic and nonirritant excipients. The use of iron oxide colorants is limited in some countries, such as the

USA, to a maximum ingestion of 5 mg of elemental iron per day. Iron oxides are accepted for use as a food additive in Europe and they are included in non-parenteral medicines licensed in many countries including Japan, UK, and USA (Rowe et al., 2009, 6th edition).

For the BHT- the antioxidant used in fill formulation of the proposed product of all strengths - optimum concentration was set to 0.10%. The ADI for the specific excipient is 0.3 mg/kg which corresponds to 18 mg /day (Pifferi et al., 2003; JEFCA, Joint FAO/WHO Expert Committee on Food Additives, 51st Technical Report Series, 2000, p. 891). All capsules of different strengths contain BHT in concentration far below the ADI for the specific excipient. Even the putative combination of more than one tablets per day - which is not recommended - to reach specific concentration for the active ingredient, thus multiplying the amount of the ingested excipients, does not reach the limits of ADI for the BHT. Hardly reaching the limit would require ingestion of ~62 capsules of 800 IU to reach a 50000 IU concentration.

Although MCT oil does not have a defined tolerable upper intake level (UL), a maximum daily dose of 50-100 grams has been suggested for improved gastrointestinal tolerance (Parrish et al., 2017). Moreover, administration of 0.5 g/kg body-weight medium chain triglycerides (35g) to healthy individuals produced no change in blood or serum triglycerides compared to subjects receiving the same dose of the long-chain triglyceride triolein (Rowe et al., 2009, 6th edition). The concentration of MCT used in the soft capsules (Table1), is far below the maximum daily recommended dose in all capsules and it remains below even in the unfortunate case of ingestion of many capsules of low strength instead of one capsule of the suggested by the doctor strength.

Even if there is no Acceptable Daily Intake (ADI) for titanium dioxide, however, an in-depth review by the European Food Safety Authority found no adverse effects in rats that consumed 2,250 mg per kg per day (NOAEL=2,250 mg/kg/day). The concentrations in the soft capsules - even in the unfortunate case of ingestion of many capsules of low strength instead of one capsule of the suggested by the doctor strength - remain far below the NOAEL reported for rats (EFSA Panel on Food Additives and Flavourings., 2019).

The use of iron oxide colorants is limited in some countries, such as the USA, to a maximum ingestion of 5 mg of elemental iron per day. The Joint Expert Committee on food additives (JECFA) set an ADI of 0-0.5 mg/kg bw. The concentrations in the soft capsules - even in the unfortunate case of ingestion of many capsules of low strength instead of one capsule of the suggested by the doctor strength - remain far below the ADI.

Thus, all the excipients used in the production of Vitamin D3 (Cholecalciferol) 800 IU, 1000 IU, 3200 IU, 20000 IU, 25000 IU, 50000 IU soft capsules, are safe and generally regarded as non-toxic in the concentrations used, always in accordance with the posology suggested in the SmPCs that accompany the products (SmPC “COLECALCIFEROL 1000 IU 3200 IU 800 IU CAPSULE, SOFT”., Sandoz; SmPC “COLECALCIFEROL 20000 IU 25000 IU 50000 IU CAPSULE, SOFT”., Sandoz).

III.5 Ecotoxicity/environmental risk assessment (ERA)

Vitamine D Will contains vitamin D3 as active substance, which is a naturally occurring vitamin. In the case of products containing vitamins, electrolytes, amino acids, peptides, proteins, carbohydrates and lipids as active pharmaceutical ingredient(s), not submitting ERA studies is justified, since due to physico-chemical nature of the API they are unlikely to result in a significant risk to the environment. An environmental risk assessment was therefore not deemed necessary.

III.6 Discussion on the non-clinical aspects

This product has been granted a market authorisation for well-established use. A non-clinical overview on the pharmacology, pharmacokinetics and toxicology has been provided, which is based on up-to-date and adequate scientific literature. The overview justifies why there is no need to generate additional non-clinical pharmacology, pharmacokinetics and toxicology data. Therefore, the member states agreed that no further non-clinical studies are required.

IV. CLINICAL ASPECTS

IV.1 Introduction

Cholecalciferol is a well-known active substance with established efficacy and tolerability. A clinical overview has been provided, which is based on scientific literature. The overview justifies why there is no need to generate additional clinical data. Therefore, the member states agreed that no further clinical studies are required.

IV.2 Pharmacokinetics

Orally taken vitamin D is absorbed through the small intestine in association with lipids, and with the aid of bile salts. It is then taken up in the lymph (Harris et al., 1999). The availability of vitamin D3 from an oily solution is generally regarded as being more effective than from dry composition solid dosage forms such as tablets (Grossmann and Tangpricha, 2010). Absorption of vitamin D3 is thought to range from 55-99 % when given in oil to stimulate bile acid release (Davies et al., 1980; Wagner et al., 2008; Goncalves et al., 2013). The presence of bile is essential for adequate intestinal absorption of vitamin D substances (Martindale, 2019).

The mechanism of absorption of non-hydroxylated species of vitamin D (such as vitamin D3) is suspected to be medicated by an unsaturable passive diffusion process. Studies on human intestinal cell line CaCO2 (Caucasian colon adenocarcinoma) and HEK (Human embryonic kidney) transfected cells clearly demonstrated the intimacy of intestinal cell membrane protein in the absorption non-hydroxylated forms at the border side of the enterocytes. Absorption of cholesterol and other lipophilic compounds (tocopherol, carotenoids) is also facilitated by these proteins which are SR-BI (scavenge receptor class B type 1), CD 36 (cluster Determinant 36) and NPC1L1 (Neimann-Pick C1- Like 1). The observations made from these proteins postulate that there is a mode shift in absorption of vitamin D from protein mediated transport to passive diffusion, depending on the concentration of vitamin D: protein mediated

transport at low concentration (dietary concentration of vitamin D) and passive diffusion at high concentration (pharmacological concentration) (Reboul et al., 2011). Further, the difference in vitamin D uptake between jejunum and duodenum clearly indicates the presence of another transporter particularly expressed in the jejunum (Goncalves et al., 2015).

Food intake potentially increases the absorption of vitamin D. One study reported the improvement of vitamin D (vitamin D2 and vitamin D3) absorption with the largest meal of day and in consequences about a 50% rise of serum level of 25(OH)D (Mulligan and Licata, 2010). The data obtained from this study could be the result of either high secretion of digestive enzymes (after heavy meal) or some specific food components (Maurya and Aggarwal, 2017).

In general lipids are most widely used vitamin D delivery medium and considered crucial for fat soluble micronutrients (Maurya and Aggarwal, 2017). Literature suggests that the species of fatty acids can affect the bioavailability of vitamin D. It has been shown that the decrease in vitamin D uptake was registered by addition of fatty acids of different chain length and degree of saturation i.e. butyric acid, octanoic acid, oleic acid and linoleic acids (Hollander et al., 1978).

Vitamin D is transported in the blood by the vitamin D binding protein (DBP, a specific binding protein for vitamin D and its metabolites in serum) to the liver (Harris et al., 1999, Christakos et al., 2010). Vitamin D3 is also stored in adipose tissue. The adipose tissue distribution is due to the lipophilic nature of vitamin D3. Jones (2008) reported the slow turnover of vitamin D3 in the body and concluded a half-life of approximately two months (Jones, 2008). Vitamin D can be stored in adipose and muscle tissue for long periods of time (Blum et al., 2008, Martindale, 2019) with vitamin D3 levels in the serum correlated to the amount of D3 in fat tissue (Blum et al., 2008).

Vitamin D3 is metabolised in the liver to 25(OH)D. 25(OH)D is metabolised in the kidneys by the enzyme 25(OH)D-1α-hydroxylase (CYP27B1) to its active form, 1,25(OH)2D (DeLuca, 2004, Holick, 2004b and 2006a). The renal production of 1,25(OH)2D is tightly regulated by plasma PTH levels and serum calcium and phosphorus levels (DeLuca, 2004; Holick, 2004b and 2006a). Fibroblast growth factor 23, secreted from the bone, causes the sodium–phosphate co-transporter to be internalised by the cells of the kidney and small intestine and also suppresses 1,25(OH)2D synthesis (Holick, 2007).

In addition, 1,25(OH)2D3 is excreted in the bile as polar metabolites, such as glucuronides and, possibly sulphates and neutral polar steroids. These compounds undergo enterohepatic recirculation in man (Kumar, 1990). Because of their high lipid solubility, cholecalciferol and its metabolites are eliminated slowly from the body. Cholecalciferol has a plasma half-life of 19 to 25 hours and a terminal half-life of weeks to months (Mawer et al., 1969; Morrow, 2001). When a single ultra-high dose of 100,000 IU of cholecalciferol was administered to healthy volunteers, the levels of 25(OH)D reached an average peak serum concentration of 42 ng/mL after 7 days from the bolus administration. Subsequently, the values of 25(OH)D decreased

steadily and it took over 50 days for the serum 25(OH)D level to drop by 50% from its peak (Ilahi et al., 2008).

The passage of vitamin D and its metabolites through the bile and hence into the intestine represents an obligatory stage for the enterohepatic circulation and fecal excretion of vitamin D metabolites by man (DeLuca, 1976; Avioli et al., 1967). Less than 4% of radioactivity from vitamin D3 appears in the urine (DeLuca 1976; Avioli et al., 1967).

Regarding hepatic insufficiency, in a clinical study the intestinal absorption and 25-hydroxylation of vitamin D in patients with primary biliary cirrhosis has been examined. The results showed that the absorption was severely impaired in test subjects but the hepatic conversion of vitamin D into 25-OH D was well preserved (Danielsson et al., 1982).

Bikle et al. (1984) studied and compared free 1,25(OH)2D levels in serum from normal subjects, pregnant subjects and subjects with liver disease. In subjects with liver disease the mean total 1,25(OH)2D concentration and the mean DBP concentration were nearly half normal values, whereas the mean free 1,25(OH)2D level was similar to normal values (Bikle et al., 1984). In conclusion, hepatic conversion of vitamin D to 25(OH)D does not seem to be severely affected in case of hepatic insufficiency and thus no dosage adjustment is recommended.

Impaired renal function can affect the metabolism of calcium, phosphate and vitamin D (Malluche et al., 2002; Bosworth and de Boer, 2013). In chronic renal failure, vitamin D metabolism and excretion are significantly impaired (Avioli et al., 1968; Bosworth and de Boer, 2013; Filipov and Dimitrov, 2019). More specifically, both renal production of 1,25(OH)2D from 25(OH)D and vitamin D catabolism are reduced in chronic kidney disease. 25(OH)D is also reduced due to increased loss in nephrotic patients (Bosworth and de Boer, 2013, Filipov and Dimitrov, 2019).

Cholecalciferol soft capsules should not be used in patients with severe renal impairment. In patients with severe renal insufficiency other forms of vitamin D should be used (Williams et al 2009, Jean et al, 2017).

IV.3 Pharmacodynamics

Cholecalciferol is the naturally occurring form of vitamin D. Vitamin D3 is a prohormone produced in skin through ultraviolet irradiation of 7-dehydrocholesterol. It is biologically inert and must be metabolised to 25(OH)D3 in the liver and then to 1,25(OH)2D3 in the kidney before function. The hormonal form of vitamin D3, i.e., 1,25(OH)2D3, acts through a nuclear receptor to carry out its many functions, including calcium absorption, phosphate absorption in the intestine, calcium mobilisation in bone, and calcium reabsorption in the kidney (DeLuca, 2004; Holick, 2016). 1,25(OH)2D3 acts via a specific nuclear vitamin D receptor (VDR) to perform its biological functions (Jones et al., 1998). Binding of 1,25(OH)2D3 to VDR initiates a cascade of macromolecular interactions ultimately leading to transcription of select target genes (Sutton and MacDonald, 2003). VDRs are distributed widely in the body: in addition to their presence in tissues involved in calcium metabolism (kidney, parathyroid glands, bone and intestine), VDRs have been identified in such locations as skin, muscle, cells of the immune

system, cells of the haematopoietic system, male and female reproductive tissues, pancreas, lung, heart and vascular tissue (Jones et al., 1998; Nagpal et al., 2005). Although this genomic pathway is responsible for most of the biological activity of vitamin D, evidence suggests that some activity may be mediated by cell surface receptors, but these are incompletely characterised and the relevance of this pathway is not universally accepted (Brown et al., 1999).

Vitamin D has been identified as one of the key nutrients that contributes to the development and maintenance of optimum bone mass. Vitamin D primarily enhances intestinal calcium and phosphate absorption and promotes bone mineralisation, thus supporting optimal skeletal growth and development from the very early life stages (Misra et al., 2008; Braegger et al., 2013). Without vitamin D, only 10 to 15% of dietary calcium and about 60% of phosphorus is absorbed (DeLuca, 2004). The interaction of 1,25(OH)2D with the VDR increases the efficiency of intestinal calcium absorption to 30 to 40% and phosphorus absorption to approximately 80% (Heaney et al., 2003; DeLuca, 2004). PTH enhances the tubular reabsorption of calcium and stimulates the kidneys to produce 1,25(OH)2D (DeLuca, 2004; Dusso et al., 2005). PTH also activates osteoblasts, which stimulate the transformation of preosteoclasts into mature osteoclasts (Holick, 2006a). Osteoclasts dissolve the mineralised collagen matrix in bone, causing osteopenia and osteoporosis and increasing the risk of fracture (Chapuy et al., 1992 and 1997; McKenna, 1992; Lips, 2001; Larsen et al., 2004; Bakhtiyarova et al., 2006; Bischoff-Ferrari et al., 2006; Boonen et al., 2006; Holick, 2006b).

Vitamin D affects calcium absorption, phosphate absorption in the intestine, calcium mobilisation in bone and calcium reabsorption in the kidney (DeLuca, 2004). Of particular importance is the regulation of the parathyroid gland by the vitamin D hormone (Jones et al., 1998).

The vitamin D hormone functions to increase serum calcium concentrations through three separate activities. First, it is the only hormone known to induce the proteins involved in active intestinal calcium absorption. Furthermore, it stimulates active intestinal absorption of phosphate. Second, blood calcium concentrations remain in the normal range even with a no-calcium diet. Two mechanisms play a role in increasing blood calcium concentrations, especially in the absence of intestinal calcium absorption. Vitamin D hormone stimulates osteoblasts to produce receptor activator nuclear factor-κB ligand (RANKL). RANKL then stimulates osteoclastogenesis and activates resting osteoclasts for bone resorption (Suda et al 2003). Therefore, the vitamin D hormone plays an important role in allowing individuals to mobilise calcium from bone when it is absent from the diet. It is very important to note, however, that in vivo both vitamin D and PTH are required for this mobilisation event. Therefore, two keys are required, similar to a safety deposit box. Third, the distal renal tubule is responsible for reabsorption of the last 1% of the filtered load of calcium, and the two hormones interact to stimulate the reabsorption of this last 1% of the filtered load. Because 7 g of calcium are filtered every day among humans, this represents a major contribution to the calcium pool. Again, both PTH and the vitamin D hormone are required. Calcium physiologic processes are such that a single low concentration of the vitamin D hormone stimulates enterocytes to absorb calcium and phosphate. If the plasma calcium concentration fails to respond, then the parathyroid glands continue to secrete PTH, which increases production of the vitamin D hormone to mobilise bone calcium (acting with PTH). Under normal

circumstances, environmental calcium is used first; if environmental calcium is absent, then internal stores are used (DeLuca, 2004).

Calcium-sensing proteins that sense plasma calcium concentrations are found in the parathyroid gland. When calcium concentrations decrease below normal, even slightly, then these transmembrane proteins, coupled to a G protein system, stimulate the secretion of PTH. PTH then proceeds to the osteoblasts and to the proximal convoluted tubule cells within seconds. Most importantly, in the convoluted tubule cells that serve as the endocrine gland for the vitamin D hormone, 1-hydroxylase concentrations are markedly elevated. This signals the vitamin D hormone, which by itself stimulates intestinal absorption of calcium or together with PTH, at higher concentrations, stimulates mobilisation of bone calcium and renal reabsorption of calcium. The increase in serum calcium concentrations exceeds the set point of the calcium-sensing system, shutting down the parathyroid gland-induced cascade of events. If the plasma calcium concentrations overshoot, then the C-cells of the thyroid gland secrete the 32-amino acid peptide calcitonin, which blocks bone calcium mobilisation. Calcitonin also stimulates the renal 1-hydroxylase to provide the vitamin D hormone for noncalcaemic needs under normocalcaemic conditions. The molecular mechanisms have not been entirely determined, except for the vitamin D hormone induction of 24- hydroxylase (CYP24) (DeLuca, 2004).

Mediated through the VDR, 1,25(OH)2D3 is essential for the development and maintenance of mineral ion homeostasis and skeletal integrity (Sutton and MacDonald, 2003; DeLuca, 2004; Holick 2004a). Amongst other functions, vitamin D is an essential component of physiological mechanisms that maintain serum calcium and phosphorus levels within a narrow range (Bouillon et al., 1998). Calcium is the primary mineral of bone and its effective absorption depends on vitamin D (Epstein, 2006). The efficiency of the absorption of renal calcium and of intestinal calcium and phosphorus is increased in the presence of 1,25(OH)2D (Dusso et al, 2005). Without vitamin D, only 10 to 15% of dietary calcium and about 60% of phosphorus is absorbed (DeLuca, 2004; Holick 2007). The interaction of 1,25(OH)2D with the VDR increases the efficiency of intestinal calcium absorption to 30 to 40% and phosphorus absorption to approximately 80% (Heaney et al., 2003; DeLuca, 2004; Heaney, 2004).

Brain, prostate, breast, and colon tissues, among others, as well as immune cells have VDRs and respond to 1,25(OH)2D, the active form of vitamin D (DeLuca, 2004; Dusso et al., 2005; Holick, 2006a). In addition, some of these tissues and cells express the enzyme 25(OH)D-1α-hydroxylase (Dusso et al, 2005; Holick, 2006a). Directly or indirectly, 1,25(OH)2D controls more than 200 genes, including genes responsible for the regulation of cellular proliferation, differentiation, apoptosis, and angiogenesis (Nagpal et al., 2005; Holick, 2006a). It decreases cellular proliferation of both normal cells and cancer cells and induces their terminal differentiation (Dusso et al., 2005; Holick, 2006a). 1,25(OH)2D is also a potent immunomodulator (DeLuca, 2004; Dusso et al., 2005) and it inhibits renin synthesis (Li, 2011), increases insulin production (Chiu et al., 2004), and increases myocardial contractility (Zitterman, 2009).

IV.4 Clinical efficacy

Treatment with daily doses

Hackman et al. (2010) performed a prospective, randomised, open-label trial to compare the efficacy and safety of a 10-day, high-dose vs a 3-month, continuous low-dose oral cholecalciferol course in a vitamin D deficient population. The primary end points were the change in serum 25(OH)D concentrations at 3 months and the development of hypercalcaemia and hypercalciuria. Fifty-nine vitamin D deficient inpatients (serum 25(OH)D < or = 50 nmol/L) were enrolled. Participants were randomly assigned to a high-dose regimen of cholecalciferol 50000 IU daily for 10 days or a 3-month, continuous low-dose cholecalciferol regimen of 3000 IU daily for 30 days, followed by 1000 IU daily for 60 days. Both groups received calcium citrate 500 mg daily. According to the results of the study, twenty-six patients completed the study within 3 - or + 1 months. The mean increases in serum 25(OH)D were similar in both the high- and low-dose groups (to 55 v 51 nmol/L, respectively; P = 0.9). There was no significant difference in the proportion of subjects who attained serum 25(OH)D concentrations > 50 nmol/L between the high- and low-dose groups (9/10 v 13/14, respectively; P = 1.0). Hypercalciuria (urine calcium > 7.5 mmol/day) occurred in three patients (two low-dose, one high-dose), while renal impairment worsened in one patient. No patient developed hypercalcaemia (corrected calcium > 2.6 mmol/L), vitamin D toxicity (25(OH)D > 200 nmol/L) or nephrolithiasis during the study. The authors concluded that both the 10-day, high dose and the 3-month, low-dose cholecalciferol regimens effectively increased serum 25(OH)D to within the normal range. The high-dose regimen may be an effective and cheap alternative for patients with vitamin D deficiency (Hackman et al., 2010).

Gallagher et al. (2012) conducted a randomised, placebo-controlled trial to determine the effect of increasing oral doses of vitamin D3 on serum 25(OH)D and serum PTH (PTH) levels in postmenopausal white women with vitamin D insufficiency (defined as a 25(OH)D level ≤50 nmol/L) in the presence of adequate calcium intake. 163 healthy postmenopausal white women with vitamin D insufficiency were enrolled and followed for 1 year. Participants were randomly assigned to receive placebo or vitamin D3, 400, 800, 1600, 2400, 3200, 4000 or 4800 IU once daily. Daily calcium supplements were provided to increase the total daily calcium intake to 1200 to 1400 mg. The primary outcomes were 25(OH)D and PTH levels at 6 and 12 months. According to the results of the study, the mean baseline 25(OH)D level was 39 nmol/L. The dose response was curvilinear and tended to plateau at approximately 112 nmol/L in patients receiving more than 3200 IU/d of vitamin D3. The RDA of vitamin D3 to achieve a 25-(OH)D level greater than 50 nmol/L was 800 IU/d. A mixed-effects model predicted that 600 IU of vitamin D3 daily could also meet this goal. Compared with participants with a normal body mass index (<25 kg/m()), obese women (≥30 kg/m(2)) had a 25-(OH)D level that was 17.8 nmol/L lower. PTH levels at 12 months decreased with an increasing dose of vitamin D3 (P = 0.012). Depending on the criteria used, hypercalcaemia occurred in 2.8% to 9.0% and hypercalciuria in 12.0% to 33.0% of participants; events were unrelated to dose. The limitation of this study was that findings may not be generalisable to other age groups or persons with substantial comorbid conditions. The authors concluded that a vitamin D3 dosage of 800 IU/d increased serum 25(OH)D levels to greater than 50 nmol/L in 97.5% of women (Gallagher et., al 2012).

Treatment with single doses

Von Restorff et al. (2009) performed a clinical case study in order to describe the changes of 25(OH)D serum concentrations achieved with a single oral dose of 300000 IU vitamin D3 among elderly rheumatology patients with severe vitamin D3 deficiency. Over a course of 4

months, they identified 33 elderly with severe vitamin D deficiency (25(OH)D<25 nmol/l) on admission to acute care. Patients were admitted for musculoskeletal pain, bone disease, or gait abnormalities. The mean age was 80.5 years (SD+/-6.1). All patients were treated with a single oral dose of 300000 IU D3 in combination with 500-1000 mg calcium supplements per day depending on their dietary calcium intake. According to the results of the study, baseline mean 25(OH)D serum concentrations were 15 nmol/l (SD+/-5.5). Mean 25(OH)D serum concentrations increased to 81.4 nmol/l (SD+/-29.7) at 3 months (29 patients) and were still 69.0 nmol/l (SD+/-17.9) at 6 months (26 patients). Mean serum calcium levels were 2.24 mmol/l (SD+/-0.11) at baseline, 2.28 mmol/l (SD+/-0.18) at 3 months, and 2.28 mmol/l (SD+/-0.13) at 6 months. Two patients with mild hypercalcaemia (2.69 mmol/l) at 3 months had normal values at 6 months. The authors concluded that a single oral dose of 300000 IU vitamin D3 raises mean 25(OH)D serum concentrations to the target mean of above 75 nmol/l at 3 months and a mean level of 69 nmol/l at 6 months. As calcium absorption is enhanced with higher 25(OH)D serum concentrations, calcium supplementation may need downward adjustment with this regimen to avoid mild hypercalcaemia (von Restorff et al., 2009).

Cipriani et al. (2010) conducted a prospective interventional study to evaluate serum changes of 25(OH)D, 1,25(OH)2D, calcium and PTH induced by a single load of cholecalciferol at 3, 15, and 30 days compared to baseline in young subjects with vitamin D deficiency. Forty-eight young subjects with vitamin D deficiency participated in the study and a single oral dose of 600,000 IU of cholecalciferol was administered to each subject. Serum changes of 25(OH)D, 1,25(OH)2D, calcium and PTH induced by a single load of cholecalciferol were evaluated. According to the results of the study, the 25(OH)D level was 15.8 ± 6.5 ng/ml at baseline and became 77.2 ± 30.5 ng/ml at 3 d ($P < 0.001$) and 62.4 ± 26.1 ng/ml at 30 d ($P < 0.001$). PTH levels concomitantly decreased from 53.0 ± 20.1 to 38.6 ± 17.2 pg/ml at 3 d and to 43.4 ± 14.0 pg/ml at 30 d ($P < 0.001$ for both). The trends were maintained in a subgroup followed up to 90 d ($P < 0.001$). Mean serum Ca and P significantly increased compared to baseline, whereas serum Mg decreased at 3 d. 1,25(OH)2D significantly increased from 46.8 ± 18.9 to 97.8 ± 38.3 pg/ml at 3 d ($P < 0.001$) and to 59.5 ± 27.3 pg/ml at 60 d ($P < 0.05$). The authors concluded that a single oral dose of 600,000 IU of cholecalciferol rapidly enhances 25(OH)D and reduces PTH in young people with vitamin D deficiency (Cipriani et al., 2010).

Comparisons of different regimes

Giusti et al. (2010) performed a randomised-controlled trial with 6-month follow-up to compare the effects of two dosing regimens of cholecalciferol on PTH and 25-hydroxy-vitamin D (25(OH)D) levels in women with secondary hyperparathyroidism (sHPTH) and hypovitaminosis D and to investigate variables affecting 25(OH)D response to cholecalciferol. The participants were sixty community-dwelling women aged 65 and older with sHPTH and hypovitaminosis D, creatinine clearance greater than 65 mL/min and without diseases or drugs known to influence bone and vitamin D metabolism. The patients were treated with cholecalciferol 300,000 IU every 3 months, once at baseline and once at 3 months (intermittent D3 group) or cholecalciferol 1,000 IU/day (daily D3 group). Serum PTH, 25(OH)D, calcium, bone-specific alkaline phosphatase, β-C-terminal telopeptide of type I collagen, phosphate, 24-hour urinary calcium excretion were measured. According to the results of the study, the two groups had similar baseline characteristics. All participants had vitamin D deficiency [25(OH)D<20 ng/mL)], and 36 subjects (60%) had severe deficiency (<10 ng/mL), with no difference between the groups (severe deficiency: intermittent D3 group, n=18; daily

D3 group, n=18). After 3 and 6 months, both groups had a significant increase in 25(OH)D and a reduction in PTH. Mean absolute increase ± standard deviation of 25(OH)D at 6 months was higher in the intermittent D3 group (22.7±11.8 ng/mL) than in the daily D3 group (13.7±6.7 ng/mL, P<0.001), with a higher proportion of participants in the intermittent D3 group reaching desirable serum concentration of 25(OH)D≥30 ng/mL (55% in the intermittent D3 group vs 20% in the daily D3 group, P.001). Mean percentage decrease of PTH in the two groups was comparable, and at 6 months, a similar proportion of participants reached normal PTH values. 25(OH)D response to cholecalciferol showed a wide variability. In a logistic regression analysis, body mass index and type of treatment appeared to be significantly associated with normalisation of 25(OH)D values. The authors concluded that cholecalciferol 300,000 IU every 3 months was more effective than 1,000 IU daily in correcting vitamin D deficiency, although the two groups achieved similar effects on PTH at 6 months. Only 55% of the higher-dose intermittent group reached desirable concentrations of 25(OH)D, suggesting that yet-higher doses will be required for adequate vitamin D repletion (Giusti et al., 2010).

Takacs et al. (2017) conducted a prospective, randomised clinical trial to compare the efficacy and safety profiles of selected daily 1000 IU, weekly 7000 IU and monthly 30000 IU vitamin D3. Sixty-four adult subjects with vitamin D deficiency [25(OH)D <20 ng/ml], were enrolled and randomised to a daily single dose of 1000 IU (group A), a once-weekly 7000 IU dose (group B) or a monthly 30000 IU dose (group C) of vitamin D3 for 3 months. Dose-responses for increases in serum vitamin 25(OH)D were statistically equivalent for each of the three groups: A, B and C. Outcomes were 13.0 ± 1.5; 12.6 ± 1.1 and 12.9 ± 0.9 ng/ml increases in serum 25(OH)D per 1000 IU, daily, weekly and monthly, respectively. The treatment of subjects with selected doses restored 25(OH)D values to levels above 20 ng/ml in all groups. Treatment with distinct administration frequency of vitamin D3 did not exhibit any differences in safety parameters. The authors concluded that the daily, weekly and monthly administrations of daily equivalent of 1000 IU of vitamin D3 provide equal efficacy and safety profiles (Takacs et al., 2017).

Toth et al. (2017) performed an open label, randomised, controlled, multicenter clinical trial to assess the safety and the efficacy of a “Slower Loading” dose of 30,000 IU vitamin D3 supplementation administered in a weekly schedule for 12 weeks in vitamin D deficient patients compared to the daily equivalent dose of 1000 IU/day regimens. 66 adult subjects (8 male/58 female) with 25OHD levels 30 ng/ml): 91% vs. 10% of subjects in after 8 weeks with 30,000 IU/wk and 1000 IU/d doses and 95% vs. 24% by end of the 12 weeks of treatment. The treatment-related increment potential was in a range of 2.26-2.92 ng/week for the weekly 30K dosing group compared to 1.32-1.70 ng/week for the 1000 IU/day standard maintenance dose group after 8 weeks. Treatment with 30,000 IU doses of Vitamin D3 in a weekly administration for 12 weeks did not abolish serum calcium levels. No difference in frequency of laboratory adverse events (AEs) and other safety parameters was observed compared to lower maintenance doses or to control group. The authors demonstrated the efficacy and safety of weekly loading oral doses of 30,000 IU vitamin D3 tablets compared to the maintenance treatment with a daily dose equivalent of 1000 IU/d, in a daily or in monthly schedule in vitamin D deficient, adult population. Weekly administration of 30,000 IU loading dose for 12 weeks does not raise safety concern, but provides an effective tool for normalisation of 25OHD levels to the desirable level of >30ng/mL in deficient patients (Toth et al., 2017).

De Niet et al. (2018) performed a monocentric, two-armed, randomised, interventional, open and parallel study to determine whether a cumulative dose of vitamin D3 produces the same effects on the serum concentration of 25(OH)D3 if it is given daily or monthly. 60 subjects with vitamin D deficiency were randomised in two different regimen treatment groups and received the same total dose of vitamin D3 supplementation over a period of 75 days followed by a period without supplementation until Day 105. The two groups of subjects were treated in parallel. They received: one tablet containing 2000 IU of vitamin D3 per day from day 1 to day 75 or two ampoules with an oily solution of vitamin D3 containing 25,000 IU on day 1, day 25, and day 50 in order to obtain the same cumulative dose after 75 days (Total dose: 150,000 IU). The 25(OH)D3 serum concentrations from baseline to day 75 were 14.3 ± 3.7 to 27.8 ± 3.9 ng/mL in the monthly group and 14.1 ± 3.4 to 28.8 ± 5.4 ng/mL in the daily group. The mean change versus the baseline level was significantly different between the groups at day 2, 4, 7, and 14 and no longer different from day 25. One day after the intake of vitamin D3, as expected, serum 25(OH)D3 and 1,25(OH)2D3 increased significantly in the monthly group, whereas they did not change significantly in the daily group. The median time to reach the 20 ng/mL target concentration was significantly different in the two groups, in favour of the monthly regimen (1 day versus 14 days; p = 0.02). The authors concluded that a monthly administration of 50,000 IU vitamin D3 provides an effective tool for a rapid normalisation of 25(OH)D3 in deficient subjects. A daily administration of the same cumulative dose is similarly effective but takes two weeks longer to reach the desirable level of 20 ng/mL (De Niet et al., 2018).

Daily dose vs single dose

Apaydin et al. (2018) conducted a randomised clinical trial to compare the effects and safety of single high-dose with daily low-dose oral colecalciferol on 25(OH)D levels and muscle strength in postmenopausal women with vitamin D deficiency or insufficiency. Sixty healthy postmenopausal women who had serum vitamin D levels 0.05). A significant increase in vitamin D levels was observed in both groups at 4 and 12 weeks after vitamin D3 treatment. The increase in the single-dose group was significantly higher than the daily low-dosage group at the 4th week (35.9 ± 9.6 ng/mL (89,6 ± 23,9 nmol/L), 16.9 ± 5.8 ng/mL (42,1 ± 14,4 nmol/L), p = 0.01). The increase in the single-dose group was significantly higher than in the daily low dosage group at the 12th week (23.4 ± 4.7 ng/mL (58,4 ± 11,7 nmol/L), 19.8 ± 7.2 ng/mL (49,4 ± 17,9 nmol/L), p = 0.049). The quadriceps muscle strength score increased significantly in the daily group at the 4th week (p = 0.038). The hamstring muscle strength score increased significantly in the daily group at the 12th week (p = 0.037). The authors concluded that although daily administration routes are more effective in improving muscle strength, a single administration is more effective in increasing vitamin D levels (Apaydin et al., 2018).

Daily dose vs individualised loading dose

Wijnen et al. (2015) performed a randomised controlled trial to compare the efficacy of an individualised cholecalciferol loading dose (LD) regimen and a daily dose (DD) regimen of cholecalciferol 800 IU in reaching 25-OH vitamin D (25OHD) levels >75 nmol/l in nursing home patients. A total of 30 nursing home patients with 25OHD levels 75 nmol/l at T 5. Secondary endpoints were the proportion of patients with 25OHD levels >75 nmol/l at T 26, safety of LD regimen, and improvement of performance tests with normalisation of vitamin D levels. According to the results of the study, median baseline 25OHD levels (interquartile range) were

comparable between the 14 DD and 16 LD patients: 20.9 (15.9-29.6) and 21.7 (16.4-32.8) nmol/l, respectively. Levels of 25OHD >75 nmol/l at T 5 were reached in 79 % of the 14 LD patients, but in none of the 13 DD patients ($p < 0.001$). At T 26, 25OHD levels >75 nmol/l were reached in 83 % of the 12 LD patients and in 30 % of the ten DD patients ($p < 0.05$). Side effects or hypercalcaemia were not observed. No improvement of performance tests was observed. The authors concluded that in nursing home patients with severe 25OHD deficiency an individualised calculated cholecalciferol LD is likely to be superior to a DD of cholecalciferol 800 IU in terms of the ability to rapidly normalise vitamin D levels (Wijnen et al., 2015).

Different loading doses

Van Groningen et al. (2010) conducted a pragmatic dose-escalation study to develop a practical cholecalciferol loading dose regimen for vitamin D-deficient adults. A total of 208 vitamin D-deficient subjects (serum 25(OH)D3) level <50 nmol/l), aged 18-88 years, were treated with solubilised cholecalciferol, 50,000 IU/ml. They received either 25,000 IU every fortnight for 8 weeks (total dose 100,000 IU), 25,000 IU every week for 6 weeks (total dose 150,000 IU) or 25,000 IU every week for 8 weeks (total dose 200,000 IU). Blood samples were collected at baseline and 10 days after the final dose of cholecalciferol. According to the results of the study, most patients were severely vitamin D deficient: 76% had a serum 25-OHD(3) level <30 nmol/l at baseline. Cholecalciferol in a cumulative dose of 100,000, 150,000, and 200,000 IU increased mean serum 25- OHD(3) level by 29 nmol/l (95% confidence interval (CI): 23-35 nmol/l), 43 nmol/l (95% CI: 36-50 nmol/l), and 69 nmol/l (95% CI: 64-75 nmol/l) respectively. The change in 25-OHD(3) (Delta25- OHD(3)) was related to the dose per kilogram body weight ($R(2)=0.38$, $P<0.0001$), and is described by the equation: Delta25-OHD(3)=0.025x(dose per kg body weight). The authors concluded that the cholecalciferol loading dose required to reach the serum 25-OHD(3) target level of 75 nmol/l can be calculated as follows: dose (IU)=40x[75-serum 25(OH)D3] x body weight (van Groningen et al., 2010).

Oral vs intramuscular administration

Tellioglu et al. (2012) performed a randomised prospective study to evaluate and compare the effects and safety of high dose intramuscular (IM) or oral cholecalciferol on 25(OH)D levels, muscle strength and physical performance in vitamin D deficient/insufficient elderly. 116 ambulatory individuals aged 65 years or older living in a nursing home were evaluated. Eligible patients with 25(OH)D levels <30 ng/ml (n=66) were randomised to IM or Oral groups according to the administration route of 600,000 IU cholecalciferol. Demographic and descriptive data were collected. Biochemical response was measured at baseline, 6th and 12th weeks. Muscle strength was measured from quadriceps by using a hand-held dynamometer and physical performance was evaluated by short physical performance battery (SPPB) at the beginning and 12th week. According to the results of the study, among the screened ambulatory elderly only 5.2% (n=6) had adequate vitamin D levels. 37.1% (n=43) were vitamin D deficient and 57.7% (n=67) were insufficient. After administration of one megadose of vitamin D, mean serum 25(OH)D levels increased significantly at 6th week (32.72±9.0 ng/ml) and at 12th week (52.34±14.2 ng/ml) compared with baseline (11.76±7.6 ng/ml) in IM group ($p<0.0001$). In Oral group levels were 47.57±12.7 ng/ml, 42.94±13.4 ng/ml and 14.87±6.9 ng/ml, respectively ($p<0.0001$). At 12th week the increase in IM group was significantly higher than Oral group ($p=0.003$). At the end of the study period, serum 25(OH)D levels were ≥30 ng/ml in all patients in IM group and in 83.3% of the patients in the Oral group. Quadriceps

muscle strength and SPPB total score increased significantly in both groups and SPPB balance subscale score increased only in IM group. Six patients (9.6%) developed hypercalciuria, no significant AEs were observed. The authors concluded that in vitamin D deficient/insufficient elderly, a single megadose of cholecalciferol increased vitamin D levels significantly and the majority of the patients reached optimal levels. Although both administration routes are effective and appear to be safe, IM application is more effective in increasing 25(OH)D levels and balance performance (Tellioglu et al., 2012).

Zabihiyeganeh et al. (2013) performed an open labeled RCT to compare the efficacy and practicality of high-dose intramuscular and oral cholecalciferol in treatment of hypovitaminosis D and to evaluate durability of the effect of each remedy. Ninety-two patients with hypovitaminosis D [serum 25(OH) D level < 75 nmol/l] were enrolled in a randomised clinical trial. Participants were randomly assigned to receive 300000 IU cholecalciferol, either intramuscularly as a single injection or orally in six divided doses during 3 months period. Serum 25(OH) D level was measured at baseline and at 3 and 6 months. According to the results of the study, both treatment regimens significantly increased the serum 25(OH)D level. Delta change in serum 25(OH) D level from baseline (presented as mean ± SEM) at month 3 was significantly higher in oral than injection group (90 ± 11·2 and 58·8 ± 8·9 nmol/l, respectively, P = 0·03); but was similar at 6th month intervention (52·1 ± 7·6 and 62·2 ± 6·7 nmol/l, respectively, P = 0·32). There was a marginally significant trend in favour of oral group in the proportion of cases attained vitamin D adequacy at 6th month (P = 0·06); but still 15% of all patients remained at < 50 nmol/l. The authors concluded that both regimens were considerably effective, safe and practical in treating hypovitaminosis D. Although they revealed superiority of oral route, at least at early short time, the way of treatment may depend on the patient's choice, compliance and availability of various forms of the drug in any regions (Zabihiyeganeh et al., 2013).

Efficacy in special populations

Ghazi et al. (2010) conducted a randomised double-blind, placebo-controlled trial to assess the efficacy and safety of different doses of vitamin D in high schoolchildren of Taleghan (latitude 36.5°N) near Tehran. 210 subjects, aged 14-20 years, 105 boys and 105 girls were assigned to three groups; group A (n=70) received 50 000 U oral cholecalciferol monthly (equal to 1600 U per day), group B (n=70), 50 000 U bimonthly (equal to 800 U/day) and group C (n=70), placebo. Serum 25(OH)D, PTH, calcium (Ca) and bone markers were measured. According to the results of the study, at baseline, girls had significantly lower concentrations of 25(OH)D than boys (19.25±16 vs 40.5±14 nmol/l). Mean 25(OH)D increased from 32±22 to 60±27.5 and 28.25±14.5 to 45.75±24 in groups A and B, respectively (P<0.001); however, it did not change over time in group C (29±18 vs 29±17.5). Increment of mean 25(OH)D was higher in group A than in group B (P<0.001). In group A, osteocalcin (OC) and bone-specific alkaline phosphatase increased (P<0.001), but in group B only OC increased (P<0.001). Urine C telopeptide and Ca did not change in all three groups; no case of hypercalcaemia was observed. The authors concluded that although monthly administration of 50000 U vitamin D3 increased serum 25(OH)D significantly, it was apparently not enough to correct vitamin D deficiency, especially in girls (Ghazi et al., 2010).

Shakiba et al. (2011) performed a randomised clinical study to specify the optimal dose of vitamin D in growing girls in Yazd during an academic year. Yazd is one the sunniest provinces

in the country and the Middle East, and, therefore, this study could reveal the minimum requirement of vitamin D in other areas with less sunshine than this region. 120 junior high school girls (aged 12-15 years) were randomly divided into 4 groups. Sixty students in groups I and II were treated for vitamin D deficiency with 300,000 IU vitamin D3 and then randomly received 50,000 U/monthly or 100,000 IU/3 months vitamin D3; 60 other students in groups III and IV received 50,000 IU/3 months and 100,000/3 months from the beginning of the academic year. Medication continued for the entire academic year; 1 month after the last dose, serum 25(OH)D levels were measured. According to the results of the study, the mean level of 25 (OH) D was 29.7 ± 4.60 ng/mL in group I and 30 ± 5.61 ng/mL in group II. Mean serum levels of 25 (OH) D were 15.2 ± 6 ng/mL and 23 ± 6.8 ng/mL for groups III and IV, respectively. The authors concluded that neither doses of about 800 IU/day nor 1000 IU/day are sufficient to maintain 25(OH)D in optimal level (> 20 ng/mL) for all, but after the treatment of deficiency, intakes of about 1000 IU/day or 2000 IU/day of vitamin D maintained optimal level in all of the students (Shakinba et al., 2011).

Black youth Vitamin D insufficiency/deficiency is commonly observed in black youth. Dong et al. (2010) conducted a randomised, blinded, controlled clinical trial to determine 25(OH)D [25(OH)D] in response to 2000 IU vitamin D supplementation over time, to evaluate the relation between 25(OH)D concentrations and total body fat mass by dual-energy x-ray absorptiometry and to determine whether vitamin D supplementation improves arterial stiffness measured by pulse wave velocity (PWV). Forty-nine normotensive black boys and girls, aged 16.3 ± 1.4 yr were randomly assigned to either the control group (400 IU/d; n = 24) or the experimental group (2000 IU/d; n = 25). According to the results of the study, plasma 25(OH)D values at baseline and at 4, 8, and 16 wk were 34.0 ± 10.6, 44.9 ± 9.4, 51.2 ± 11.1, and 59.8 ± 18.2 nmol/liter, respectively, for the control group; and 33.1 ± 8.7, 55.0 ± 11.8, 70.9 ± 22.0, and 85.7 ± 30.1 nmol/liter, respectively, for the experimental group. The experimental group vs. the control group reached significantly higher 25(OH)D concentrations at 8 and 16 wk, respectively. Partial correlation analyses indicated that total body fat mass at baseline was significantly and inversely associated with 25(OH)D concentrations in response to the 2000-IU supplement across time. Furthermore, carotid-femoral PWV increased from baseline (5.38 ± 0.53 m/sec) to posttest (5.71 ± 0.75 m/sec) in the control group (P = 0.016), whereas in the experimental group carotid-femoral PWV decreased from baseline (5.41 ± 0.73 m/sec) to posttest (5.33 ± 0.79 m/sec) (P = 0.031). The authors concluded that daily 2000 IU vitamin D supplementation may be effective in optimising vitamin D status and counteracting the progression of aortic stiffness in black youth. Plasma 25(OH)D concentrations in response to the 2000 IU/d supplementation are negatively modulated by adiposity (Dong et al., 2010).

Pappa et al. (2012) conducted a randomised, controlled clinical trial to compare the efficacy and safety of three vitamin D repletion regimens for treating vitamin D insufficiency [serum 25(OH)D concentration less than 20 ng/ml] among children with inflammatory bowel disease. The study was not blinded to participants and investigators. Eligibility criteria included diagnosis of IBD, age 5-21, and serum 25OHD concentration below 20 ng/ml. 61 patients completed the trial and two withdrew due to AEs. Patients received orally for 6 wk: vitamin D2, 2,000 IU daily (arm A, control); vitamin D3, 2,000 IU daily (arm B); vitamin D2, 50,000 IU weekly (arm C); and an age-appropriate calcium supplement. The change in serum 25OHD concentration (Δ25OHD) (ng/ml) wase measured. Secondary outcomes included change in serum intact PTH concentration (ΔPTH) (pg/ml) and the AE occurrence rate. According to the

results of the study, after 6 wk, Δ25OHD ± se was: 9.3 ± 1.8 (arm A); 16.4 ± 2.0 (arm B); 25.4 ± 2.5 (arm C); P (A vs. C) = 0.0004; P (A vs. B) = 0.03. ΔPTH ± SE was -5.6 ± 5.5 (arm A); -0.1 ± 4.2 (arm B); -4.4 ± 3.9 (arm C); P = 0.57. No participant experienced hypercalcaemia or hyperphosphatemia, and the prevalence of hypercalciuria did not differ among arms at follow-up. The authors concluded that oral doses of 2,000 IU vitamin D3 daily and 50,000 IU vitamin D2 weekly for 6 wk are superior to 2,000 IU vitamin D2 daily for 6 wk in raising serum 25OHD concentration and are well-tolerated among children and adolescents with inflammatory bowel disease. The change in serum PTH concentration did not differ among arms (Pappa et al., 2012).

Harel et al. (2011) conducted a retrospective chart review of male and female adolescents who suffered from obesity and who had been screened for vitamin D status and lipid abnormalities to examine the effect of management of low vitamin D status in obese adolescents. Vitamin D deficiency was defined as 25(OH)D level of 30 ng/mL. Adolescents with vitamin D deficiency were treated with 50,000 IU of vitamin D once a week for 6-8 weeks, whereas adolescents with vitamin D insufficiency were treated with 800 IU of vitamin D daily for 3 months. Repeat 25(OH)D was obtained after treatment. According to the results of the study, although there was a significant ($p < .00001$) increase in mean 25(OH)D after the initial course of treatment with vitamin D, 25(OH)D levels normalised in only 28% of the participants. Repeat courses with the same dosage in the other 72% did not significantly change their low vitamin D status. The authors concluded that increased surveillance and possibly higher vitamin D doses are warranted for obese adolescents whose total 25(OH)D levels do not normalise after the initial course of treatment (Harel et al., 2011).

Radhakishun et al. (2014) conducted a retrospective cohort study to test the efficacy and tolerability of a high loading dose vitamin D3 supplementation of 25,000 IU weekly in multiethnic obese children, 8-18 years of age, with vitamin D insufficiency/deficiency. Fasting blood samples were drawn for the assessment of vitamin D. Vitamin D-insufficient/-deficient children (50 nmol/l) was reached in >75% without side effects nor reaching toxic levels. According to the results of the study, in total, 109 children (mean ± SD age 11.1 ± 3.0, 34.2% boys, 90.8% obese) received vitamin D supplementation. In 84.4% of the children, the vitamin D status improved from insufficiency/deficiency (<50 nmol/l)) to sufficiency (≥50 nmol/l). The majority of children that did not reach vitamin D sufficiency reported non-compliance. No side effects were reported, and the highest level reached was far below the threshold for toxicity. The authors concluded that a high loading dose vitamin D3 supplementation is effective and well-tolerated in this cohort of multiethnic obese children with vitamin D insufficiency/deficiency (Radhakishun et al., 2014).

IV.5 Clinical safety

Vitamin D has a good safety profile (Hathcock et al., 2007). The evidence is clear that vitamin D toxicity is one of the rarest medical conditions and is typically due to intentional or inadvertent intake of extremely high doses of vitamin D (usually in the range of >50,000-100,000 IU/day for months to years) (Hossein-nezhad and Holick, 2013; Holick, 2015). A large 10-year population-based study in a northern population provided evidence that the incidence of 25(OH)D values >50 ng/mL increased dramatically from 2002-2011, without a corresponding increase in acute clinical toxicity. Most cases of 25(OH)D levels >100 ng/mL

were associated with prolonged use of high-dose vitamin D supplements, which are available without a prescription. Based on the results of the large, 10-year population-based study conducted by Dudenkov et al. (2015), caution and monitoring serum 25(OH)D and calcium was only recommended in the case of using intermittent vitamin D doses ≥50,000 IU (or daily doses ≥4,000 IU) on a continued basis (Dudenkov et al., 2015). Daily vitamin D supplementation is often inadequate in treating vitamin D deficiency due to poor compliance. A single, large dose of vitamin D given at timed intervals may be an alternative strategy. Few studies have documented complications following high-dose vitamin D supplementation. However, vitamin D doses >500 000 IU should be used judiciously in order to minimise AEs (Kearns et al., 2014).

IV.6 Risk Management Plan

The MAH has submitted a risk management plan, in accordance with the requirements of Directive 2001/83/EC as amended, describing the pharmacovigilance activities and interventions designed to identify, characterise, prevent or minimise risks relating to Vitamine Will D. At the time of approval, the most recent version of the RMP was version 1.1 dated 21 June 2023.

Table 1. Summary table of safety concerns as approved in RMP

Important identified risks	None
Important potential risks	None
Missing information	None

The member states agreed that routine pharmacovigilance activities and routine risk minimisation measures are sufficient for the risks and areas of missing information.

IV.7 Discussion on the clinical aspects

This procedure concerns a well-established use application for cholecalciferol. For this authorisation, reference is made to literature. No new clinical studies were conducted. The pharmacokinetics of cholecalciferol can be considered well established. The bridge to the products used in the literature to claim WEU is established as adequate justification has been provided by the MAH. Risk management is adequately addressed. The clinical aspects of this product are approvable

V. USER CONSULTATION

The package leaflet (PL) has been evaluated via a user consultation study in accordance with the requirements of Articles 59(3) and 61(1) of Directive 2001/83/EC. The language used for the purpose of user testing the PL was English.

A user consultation with target patient groups on the package leaflet (PL) has been performed on the basis of a bridging report making reference to Colecalciferol, PL 20491/001 UK for the content and to Rabeprazole, PT/H/0881/01-02/DC for the layout. The bridging report

submitted by the MAH has been found acceptable; bridging is justified for both content and layout of the leaflet.

VI. OVERALL CONCLUSION, BENEFIT/RISK ASSESSMENT AND RECOMMENDATION

Vitamine Will D 800 IU, 1000 IU and 3200 IU soft capsules have a proven chemical-pharmaceutical quality. Vitamine Will D is an effective drug, which is considered widely established. The benefit/risk balance is considered positive. Bridging has been shown to be in compliance with the requirements of European guidance documents.

The Board followed the advice of the assessors.

There was no discussion in the CMD(h). Agreement between member states was reached during a written procedure. The member states, on the basis of the data submitted, considered the well-established use has been demonstrated for Vitamine Will D with the reference product, and have therefore granted a marketing authorisation. The decentralised procedure was finalised with a positive outcome on 30 August 2023.

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STEPS TAKEN AFTER THE FINALISATION OF THE INITIAL PROCEDURE - SUMMARY

Procedure number	Scope	Product Information affected	Date of end of procedure	Approval/ non approval	Summary/ Justification for refuse
NL/H/4946/003-5/IA/006/G	Change in test procedure for the finished product - Minor changes to an approved test procedure Submission of a new or updated Ph. Eur. certificate of suitability or deletion of Ph. Eur. certificate of suitability: For an active substance, For a starting material/reagent/intermediate used in the manufacturing process of the active substance, For an excipient - European Pharmacopoeial TSE Certificate of suitability for an active substance/starting material/reagent/ intermediate/or excipient - New certificate for a starting material/reagent/ intermediate/or excipient from a new or an already approved manufacturer	No	20-10-2023	Approved	N/A
NL/H/4946/003-5/IB/010/G	Change in the shelf-life or storage conditions of the finished product - Extension of the shelf life of the finished product - As packaged for sale (supported by real time data) Change in the shelf-life or storage conditions of the finished product - Change in storage conditions of the	Yes	30-04-2025	Partially approved	N/A

	finished product or the diluted/reconstituted product				