2.4 Non-clinical overview

2.4.1 Overview of the nonclinical strategy

A Marketing Authorization application for Cyanocobalamin 50 mcg Tablets is being filed. The products Cyanocobalamin 50 mcg Tablets is generic product of the proprietary medicinal product Cytacon 50 micrograms Tablets containing Cyanocobalamin 50 micrograms manufactured Mercury Pharma Group Ltd, Capital House, 85 King William Street, London EC4N 7BL,UK for which the marketing authorization was granted in the European Union.

Vitamin B12 (or cyanocobalamin, CNCbl) was discovered exactly 60 years ago in the laboratories of [redacted] as the anti-pernicious anemia factor, and its structure was revealed a few years later by the crystallographic studies in the [redacted] laboratory. B12 is one of the most complex cofactors in nature and belongs to the family of tetrapyrrolic-derived macrocyclic compounds. Five of the six ligands to the central cobalt atom in free B12 are endogenous, being provided by the corin ring itself. In CNCbl, the sixth ligand, which occupies the upper axial position, is a cyano group. Although the biological origin of the cyano group in vitamin B12 is unknown, it is the cofactor form commonly used in multivitamin

[redacted]

The history of vitamin B12 (cobalamin, Cbl) originated with the descriptions of a progressive macrocytic anaemia, made independently by [redacted] in the nineteenth century. At that time, [redacted] described also spinal cord lesions that were associated with haematological changes typically seen in macrocytic anaemia. However, it was not until [redacted] detected the anti-pernicious anaemia factor provided by crude liver homogenates and red meat in the diet that the role of this factor in haematological disorders became evident, and focus was directed towards its further identification. A research hunt spanning over 2 decades led independently to the isolation of the pure anti-pernicious anaemia factor, which dramatically changed the therapeutic solutions for pernicious anaemia from several grams of uncooked liver to a few micrograms of a red crystalline compound named vitamin B12 or Cbl. In 1956 [redacted] resolved the complex structure of the Cbl molecule by X-ray crystallography (5),

and a few years later the coenzyme function of the vitamin in bacterial glutamate metabolism was discovered in [redacted] laboratory. They isolated and partly described an orange crystalline compound that was termed 5-deoxyadenosylcobalamin (AdoCbl) (6). The significance of AdoCbl as cofactor for methylmalonyl-CoA mutase involved in propionate metabolism was demonstrated shortly after (7, 8). This was followed by the discovery of the other biologically active Cbl derivative, methylcobalamin (MeCbl), which was isolated both from liver and blood cells of humans and from bacteria (9). It was found to act as cofactor for mammalian methionine synthase (5-methyltetrahydrofolate homocysteine methyltransferase, MS, EC 2.1.1.13) in the methyl transfer reaction from homocysteine (Hcy) to form methionine (Met). The structure of AdoCbl was exactly identified by X-ray analysis in 1966 (11), whereas the three-dimensional structure of MeCbl was first solved almost two decades later (12).

[redacted]

An abridged dossier is being submitted for grant of marketing authorization in accordance with article 10.1 of Council Directive 2001/83/EEC as amended. No new non-clinical study is conducted by the applicant. No new non-clinical study is conducted by the applicant. This non-clinical overview is based on the data obtained from international published articles, books and internet, typically searching databases of regulatory authorities i.e. approved monographs, pub med databases, science direct databases and cross-references through Google search.

2.4.2 Pharmacology

In-vitro

The objective of this study was to assess the in vitro fertilization (IVF) of pig oocytes using frozen-thawed boar sperm in media supplemented with Cyanocobalamin. Frozen semen pellets were thawed and incubated for 1 h in fertilization media containing Cyanocobalamin (0, 0.5, 1.0 or 2.0 μm) and evaluated for forward progressive motility, viability, and embryo cleavage. Forward progressive motility of the 0.5 and 1.0 μm Cyanocobalamin supplements was higher ($P < 0.05$) than the 0 and 2.0 μm cyanocobalamin supplements. Membrane viability of sperm

supplemented with 0.5 µm cyanocobalamin was higher ($P < 0.05$) than all other groups. Oocytes were matured and fertilized with frozen-thawed boar semen that was previously incubated for 1 h in fertilization media containing Cyanocobalamin (0 or 0.5 µm; 100 oocytes/treatment). Fertilization characteristics were evaluated 12 h after IVF of oocytes and embryo development was analyzed at 48 h and 144 h post-IVF. There were no significant differences between treatment groups when evaluating fertilization, polyspermic penetration or male pronucleus development. Embryos derived from oocytes fertilized with 0.5 µm cyanocobalamin supplemented sperm had a higher percentage ($P < 0.05$) of cleaved embryos compared to those without Cyanocobalamin supplementation at 48 h after IVF. There were no significant differences in the percentages of embryos reaching the blastocyst stag 144 h after IVF between treatment groups. The results of this study suggest that there are positive effects of 0.5 µm Cyanocobalamin supplementation during the incubation of frozen-thawed boar semen on early development of IVF derived pig embryos.

[redacted]

In-vivo

The antinociceptive effect of cyanocobalamin (Vit B12) has been reported in animal models and human studies. Our previous study showed the effect of Vit B12 on morphine tolerance. The dependence and tolerance were induced in male mice using subcutaneous morphine injections, 3 times a day (50, 50 and 75 mg/kg/day) for 3 days. Mice also received Vit B12 (100, 250 and 500 µg/kg), clonidine, memantine and saline intraperitoneally before morphine administration. On fourth day mice received only 7 mg /kg morphine just before tail-flick test. To determine the expression of morphine dependence and tolerance, all compounds were injected once intraperitoneally on the day of experiment. The tolerance was evaluated by the tail-flick test. The effect of Vit B12 and other agents on dependence were evaluated by counting the number of jumps (induced by naloxone 5 mg/kg). Co-administration of Vit B12 (100-500 µg/kg) and morphine in 3 days reduced the development of tolerance to morphine analgesic effect (8.2±0.5

and 7.83±0.5 s. vs. normal saline, 3.57±0.3 s). Repeated administration of Vit B12, also, diminished the reduced naloxane withdrawal signs of naloxone withdrawal test (100-500 µg/kg: 5±1.9 and 1.2±0.8 jumps vs. normal saline 72.6±12.2). However, Vit B12 had no effect on the expression of morphine tolerance and physical dependence. It is concluded that co-administration of Vit B12 and morphine could reduce tolerance to analgesic effect of morphine chronic administration and also reduce its withdrawal symptoms.



In this study, the anti-nociceptive and anti-inflammatory effects of Cyanocobalamin (Vit B12) against acute and chronic pain and inflammation were evaluated in mice. Vit B12 (0.87, 1 and 1.77 mg/kg) were injected intraperitoneally. The anti-nociceptive effects against acute pain were examined using hot-plate and writhing tests. The chronic pain was examined 14 days after sciatic nerve ligation using the hot-plate test. Morphine (10 mg/kg) was used as a positive control. Anti-inflammatory effects of Vit B12 against acute and chronic inflammation were assessed using xylene-induced edema in ears and granuloma caused by compressed cotton implantation, respectively. In these tests, sodium diclofenac (15 mg/kg) was used as a positive control. Vit B12 showed a dose related effect in acute anti-nociceptive test and increased the anti-nociceptive effect of morphine in chronic treatment. Vit B12 demonstrated an anti-nociceptive effect in chronic studies as single or continues daily treatment and increased significantly the anti-nociceptive effect of morphine. All doses of Vit B12 significantly decreased xylene-induced ear edema. Maximum anti-inflammatory effect (37.5%) was obtained at dose of 1 mg/kg. In chronic inflammation, Vit B12 significantly decreased granuloma formation in mice. In conclusion our work presents some experimental evidence supporting the administration of cyanocobalamin in controlling acute and chronic neuropathic pain. Cyanocobalamin may have anti-inflammatory effect. It may reduce tolerance to anti-nociceptive effect of morphine as well.

[redacted]

A study was conducted to compare the effect of selected drugs, Intellan and cyanocobalamin on some aspects of animal behavior, like effect on memory, depression, gross behavior and exploratory activity. Results of present investigations reveals cyanocobalamin to be a good memory enhancer and intellan also showed memory boosting effects in addition to anti-anxiety activity which affects overall performance.

[redacted]

A study was conducted to explore the effect of khamira-e-gao zaban amberi jawahirdar and the results were compared with the standard drug Cyanocobalamin (vitamin B12). Different CNS parameters had been performed on mice. The drugs were administered to mice and results were observed on day 20 and30. The dose of khamira administered to one group was 0.1 ml containing 0.83 mg dissolved in milk (9 mg dissolved in 10 ml of milk). While 0.04 ml of Cyanocobalamine was given to another group, third group serves as control. The results showed decrease in the time taken to reach the platform in stationary rod by both drugs, while exploratory activities was also found increased in head dip, cage crossing and open field activities. It is concluded from our findings that khamira-e-gao zaban amberi jawahir dar and Cyanocobalamin posses marked effect on behavior as well as it also possess memory enhancing effect.

[redacted]

In this study a herbal product Intellan containing Centella asiatica, Bacopa monniera, Coriandum sativum, Amomum subulatum, Emblica officinal is and another product Cytacon (Cyanocobalamin) were selected. The study was designed on animal models to explore the effects on different parameters. For this the animals were given chronic dosing for 6-8 weeks during and after which the parameters were observed to determine their effects. The purpose of focusing on such formulations is to do hematological screening in long-term use. The hematological parameter included hemoglobin/HCT, total leucocyte count, platelets. The lymphocytes and the monocytes counts were increased significantly by intellan, while Cyanocobalamin increases RBC counts, platelet counts, monocyte counts, hematocrit etc significantly. The SGPT, SGOT were found increased in both of these drugs.

[redacted]

2.4.3 Pharmacokinetics

2.4.3.1 Absorption

A study was conducted by taking Twenty-four adult mongrel dogs of both sexes, ranging in weight from 6 to 17.5 kg. They were maintained in the kennels on a mixed commercial cereal-base diet supplemented with fresh meat. The dogs were fasted for 16 to 24 hours prior to initiating the experimental procedures. They were anaesthetized with intravenous injections of nembutal (30mg per kilogram of body weight). Supple mentary doses of nembutal were given during the experimental period to maintain light anaesthesia and 5 to 10 ml of saline were injected subcutaneously every 30 minutes to prevent dehydration. Solutions of crystalline Cyanocobalamin or saline in 5- to 10-ml volumes were placed in the stomach or duodenum by means of a rubber tube passed through the esophagus. When solutions were placed in the stomach, a ligature just prior to the pyloric sphincter prevented the administered solution from entering the duodenum. In some experiments, the solutions were placed in 4-inch segments of duodenum between ligatures placed at the oral and aboral positions. In other experiments, solutions were placed in the unligated duodenum. Heparinized blood samples were obtained

from the jugular and from the portal vein prior to and at intervals following the administration of Cyanocobalamin or saline. In experiments where urine was required, 4-hour collections were obtained by bladder catheterization (with the dogs under anaesthesia) prior to and following the administration of the vitamin. The collected urine was adjusted to pH 6.8Â ± 0.1 and frozen until analyzed.

In the post-absorptive state, plasma vitamin B12 activity of dogs is the same in portal and peripheral blood plasma, but portal plasma contains greater amounts of activity than does peripheral plasma during active absorption of the vita min from the intestinal tract. Crystalline vitamin B12placed in ligated segments of the duodenum or in the unligated duodenum of dogs results in the rapid appearance of vitamin B12activity in the plasma of portal and peripheral blood. During absorption, small butn definite amounts of vitamin activity also appear in the urine.

In dogs subjected to operation but administered saline in place of vitamin B12, plasma and urine activity remained constant. The plasma vitamin B12activity of dogs with stomachs ligated at the duodenal cap remained essentially constant following the administration of vitamin.

The major portion of vitamin B12administered by intra venous injection, in an amount approximately that found in plasma during oral absorption, results in a rapid elimination of the vitamin in the urine. These data are discussed in relation to the problem of vitamin B12 absorption in man and animals.

[redacted]

To investigate the fate of intrinsic factor and cobalamin during cobalamin absorption, we incubated enterocytes isolated from guinea pig ileum for periods of up to 30 min with ^{57}Co-labeled Cyanocobalamin bound either to human intrinsic factor or to rabbit intrinsic factor biosynthetically labeled with [^{35}S] methionine. When the labeled complex was incubated for 30 min with isolated ileal cells under conditions that block cellular metabolism, virtually all cellular radioactivity could be removed by washing the cell surface with EDTA or acid. In contrast, washing removed only half the radioactivity from cells incubated at 37^0C in 0_2 When residual cellular radioactivity was extracted and analyzed by gel filtration, 80-94% of both the ^{35}S and

^{57}Co radioactivity elutedin the same fractions as the original complex. The remaining 6-20% eluted as free [^{57}Co] cobalamin or [^{35}S] methionine. To examine events occurring after 30 min, we instilled into tied-off ileal loops of intact guinea pigs radio labeled intrinsic factor-cobalamin complex and extracted nondissociable radioactivity 2-4.5h later. The proportion of extracted ^{57}Co eluting as free cobalamin increased to 39-46%, that eluting as intrinsic factor-cobalamin complex declined to 22-45%, and 9-34% now eluted as a macromolecule that reacted with antitrans cobalamin II antibody but not antiintrinsic factor antibody. Extracted 35S radioactivity eluted in several peaks in addition to the intrinsic factor peak. These findings suggest that (a) after reversible attachment of intrinsic factor-cobalamin complex to its ileal surface receptor, an energy-dependent process prevents removal of the complex from the cell surface by EDTA or acid; (b) cobalamin dissociates from intrinsic factor and, as suggested by previous workers, binds to a molecule antigenically similar to trans cobalamin II; and (c) intrinsic factor is slowly degraded and forms breakdown products that are detectable in ileal extracts.

[redacted]

A study was conducted nineteen rabbits, ten males and nine females, aged between 9 and 12 months, were used for the experiments. Some animals were used more than once, after the entire isotope from previous doses had been excreted. The rabbits were fed without restriction on a diet of oats and hay.

For the experiments the animals were kept in separate galvanized iron cages with metal grid floors and devices for separating urine from faeces. In most experiments coprophagy was prevented by collars similar to those used in our earlier work [redacted]

The collars were put in place about 18 h before the [^{58}Co] Cyanocobalamin was given, and the animals were starved overnight before administering the dose and for 6 h afterwards.

The quantities of Cyanocobalamin given ranged from 0-055 to 150 pg. For doses of 0.055 and 0.1 pg, [58Co] Cyanocobalamin of specific activity 18 pc/,ug (Radiochemical Centre, Amersham, Bucks) was used. For the larger doses, I pg (approximately I pc) of [~Co] Cyanocobalamin was mixed with the appropriate amount of non-radioactive Cyanocobalamin (Cytamen ; Glaxo Laboratories Ltd). The Cyanocobalamin was administered by stomach tube in

about 4 ml water and was washed into the stomach with a further 10-15 ml water. Faeces were collected for each period of 24 h until 2 successive days' faecal collections each contained less than 1 % of the radioactivity in the dose; urine was collected similarly until 2 successive days' collections each contained less than 0.01 % of the dose.

[redacted]

2.4.3.2 Distribution

Fifteen rats were fed on a diet containing different concentrations of B12 supplemented with s.c. injections of B12. Twenty four hours prior to sacrifice, all animals were injected with [^{57} Co] B12. The vitamin contents of kidneys, liver, spleen, brain, testis, intestine, skeletal muscle, serum and urine were analysed. Both total tissue vitamin B12 accumulation and [^{57}Co] B12 were determined to compare steady-state B12 and the distribution of an acutely injected dose. In the kidney, free and protein-bound B12 was determined by gel filtration.

The rat kidneys accumulated more B12 during normal and loaded conditions than any other tissue. A 110-fold increase in vitamin content was observed from the deficient to the loaded conditions in the kidney compared with a 3.5-fold increase in the liver. In contrast to all other organs, significantly smaller amounts of acutely injected B12 accumulated in the kidneys in the vitamin-deprived state compared with both the normal and the vitamin-loaded condition.

The present study suggests a significant role for the rodent kidney in vitamin B12 metabolism. We propose a model for rat tissue uptake consistent with the presence of two different TC–B12 receptors and renal uptake following filtration of TC– B12 in the glomeruli. The presented model allows for the reduced renal uptake and accumulation in vitamin deprived conditions, thus reserving the vitamin for other tissues, including nerve tissue and bone marrow, which are more sensitive to vitamin B12 deficiency.

[redacted]

2.4.3.3 Metabolism and excretion

Vitamin Bl2 (hydroxycobalamin) is endocytosed by mammalian cells as a complex with transcobalamin I1 and then processed to free BIZ in lysosomes. The mechanism by which free

BIZ becomes available for subsequent cellular metabolism has been uncertain. Lysosomal transport of Cyanocobalamin (BIZ) was examined using membrane vesicles prepared from Percoll gradient purified lysosomes. BIZ uptake by vesicles was dependent upon pH and was inhibited by the protonophore CCCP. Transport exhibited saturation kinetics with a K, of 3.5 WM and temperature dependence with a Ql0 of 1.8. Uptake of BIZ was dependent upon divalent cations and was inhibited by EDTA. Preparation of vesicles in the presence of 100 p~ BIZ resulted in stimulation of uptake consistent with a mechanism of counter transport. Excess Cyanocobalamin, adenosylcobalamin, methylcobalamin, or cobinamide dicyanide inhibited uptake of BIZ. Trans-stimulation studies showed that only the first three compounds are actually transported species with Cyanocobalamin as the preferred substrate. We conclude that lysosomes have a specific transport system for vitamin BIZ that results in release of this enzyme cofactor to the cytoplasm.

[redacted]

A study was conducted to examine the ability of hydroxocobalamin (vitamin B12a, HO-Cbl) to counteract the toxicity of cyanide by promoting the renal excretion of cyanide anion, we studied the pharmacokinetics of HO-Cbl and Cyanocobalamin (vitamin B12, CN-Cbl) given in high doses to dogs. After administration of intravenous bolus doses of HO-Cbl and CN-Cbl (5, 10, 25 mg/kg) to female foxhounds, peak plasma concentrations were attained within 1 minute, after which the cobalamins disappeared from plasma in a manner consistent with two-phase distribution and elimination kinetics. While mean t1/2 measurements for the initial distribution phase were similar for HO-Cbl and CN-Cbl, the secondary excretion phase was more prolonged for HO-Cbl than for CN-Cbl (t1/2, 94.75 minutes vs 62.64 minutes, respectively). Pharmacokinetic data permitted the design of a HO-Cbl infusion schedule that resulted in stable plasma concentrations in test animals. When NaCN was then given intravenously to animals preloaded with HO-Cbl (20 micrograms/ml plasma), conversion of HO-Cbl to CN-Cbl in vivo, was documented by both thin-layer and high-performance liquid chromatographic analysis of successive urine samples. Moreover, in dogs preloaded with HO-Cbl (20 micrograms/ml plasma), up to 10% of the cyanide administered as NaCN infusions was recovered in urine as

CN-Cbl by 60 minutes, compared with less than 0.3% in dogs not treated with HO-Cbl. In addition, under these conditions of HO-Cbl loading, the minimum doses of NaCN required to produce sublethal signs of cyanide toxicity (hypotension, tachypnea, tachycardia) were found to be increased to levels that in some cases exceeded normally lethal doses.

[redacted]

Both hydroxocobalamin and Cyanocobalamin are excreted mainly via the kidneys in dogs. In human volunteers, hydroxocobalamin was also excreted mainly via the renal route (58-74% of total clearance). The mean half-lives for 'free' cobalamins were 3h in the rat, 6h in the dog and 28h in humans. For Cyanocobalamin, the half-lives were 0.3 to 0.5h in rats, 0.8 to 1h in dogs and 9.3 ± 3.2 hours in humans.

[redacted]

2.4.4 Toxicology

2.4.4.1 Acute Toxicity

Spirulina Pacifica as a Source of Cobalamin Vitamin B-12 has been subjected to extensive safety studies, independent feeding studies in France, Mexico and Japan demonstrated no undesirable results or toxic effects on humans, rats, pigs, chickens, fish and oysters. There have been no negative effects reported for acute toxicity, chronic toxicity or reproduction [redacted]

2.4.4.2 Repeat dose studies

Hydroxocobalamin has a low acute toxicity. The main target organs of toxicity in repeat-dose studies were the liver, kidney and bone marrow, with findings suggestive of tissue overload with high quantities of hydroxocobalamin. Similar findings were seen in a repeated dose study with Cyanocobalamin.

Cyanocobalamin, the reaction product of hydroxocobalamin and cyanide was administered intravenously to dogs for 2 weeks (40, 100 and 400 mg/kg/day). Kidney findings consistent with an overload phenomenon, and bone marrow finding similar to those reported with hydroxocobalamin were observed at the high dose level. Considering this NOAEL in dogs and

Cyanocobalamin levels in intoxicated victims treated with hydroxocobalamin, safety factors based on Cmax ranged from 1 to 3.

[redacted]

2.4.4.3 Chronic studies

This paper reports the bodily distribution of total cobalamin and individual cobalamins at the termination of an experiment on the effects of a low cobalamin diet and chronic cyanide or thiocyanate administration in baboons. The results show that the distribution of cobalamins in the tissues of the baboon can be altered by a low cobalamin diet and also by chronic intoxication with cyanide, whether or not the animals are on a low cobalamin diet. All animals on the low cobalamin diet showed a reduction in total and individual cobalamins. In blood plasma and erythrocytes, kidney, spleen, testis and brain, the proportion of methylcobalamin tended to be disproportionately reduced in cobalamin-depleted animals. This reduction was lessened or prevented by the administration of cyanide. Neither cyanide not thiocyanate produced a significant increase in the proportion of Cyanocobalamin in plasma, though thiocyanate produced a large increase in Cyanocobalamin in erythrocytes. In liver, Cyanocobalamin was more than doubled by the administration of cyanide to cobalamin-depleted animals.

[redacted]

Convulsions, followed by cardiac or respiratory failure, have been described in mice given 1.5-3 mg/kg body weight of vitamin B12; clinical signs were not thought to be due to hypersensitivity.

[redacted]

2.4.5 Overview of Safety

Cyanocobalamin 50 mcg Tablets is a well-established and has an acceptable level of safety in the indications approved for the reference product, Cytacon 50 micrograms Tablets containing Cyanocobalamin 50 micrograms manufactured Mercury Pharma Group Ltd, Capital House, 85 King William Street, London EC4N 7BL,UK for which the marketing authorization was granted in the European Union.

No toxic effects of B12 have been encountered in human or animal at any level of oral intake [redacted] The overall evidence indicates that

vitamin B12 is virtually nontoxic. Doses of 1,000 µg per day were administered to a child by intravenous injection for a year without adverse effect [redacted] Even if 100 percent metabolic liberation from Cyanocobalamin is assumed, the cobalt and cyanide contributions of 1000 µg of vitamin B12 are toxicologically insignificant.

[redacted]

Carcinogenesis, Mutagenesis

Impairment of Fertility

No long-term animal studies have been performed to evaluate carcinogenic or mutagenic potential, or whether this drug affects fertility of males or females.

Pregnancy Category C

Adequate reproduction studies have not been performed in animals to determine whether this drug affects fertility in males or females, has teratogenic potential, or has other adverse effects on the fetus. This drug should be used in pregnant women only when clearly needed.

[redacted]

No systematic toxicological studies have been reported for vitamin B12. There are no reports attributing carcinogenic or mutagenic or teratogenic potential to Cyanocobalamin [redacted] In one study a tumour promoting effect was reported in a rat model, but this study is not considered relevant for safety assessment in humans.

There are also no adverse effects known for vitamin B12 from foods, or from supplements in amounts far in excess of needs. Some studies suggested acne formation after high parenteral doses of hydroxocobalamin, but not with cyanocobalamin, or after a combination of vitamins A, B6 and B12 given orally.

Oral and parenteral supplementation with dosages between 1-5 mg every fortnight or month have been given for long periods, up to at least 5 years, to patients with compromised vitamin B12 absorption, without any identified adverse effects. It should be noted, however, that these studies were not designed to find adverse effects.

Therefore there are no clearly defined adverse effects produced by vitamin B12 that can be used to define a LOAEL or NOAEL, which can be used as a basis for deriving an UL.

[redacted]

2.4.6 Integrated overview and conclusions

Vitamin B12 (or Cyanocobalamin, CNCbl) was discovered exactly 60 years ago in the laboratories of [redacted] as the anti-pernicious anemia factor, and its structure was revealed a few years later by the crystallographic studies in the [redacted] laboratory. B12 is one of the most complex cofactors in nature and belongs to the family of tetrapyrrolic-derived macrocyclic compounds.

Cyanocobalamin is a synthetic form of vitamin B 12 with vitamin B 12 activity. It is the most stable and widely used form of vitamin B 12 and has hematopoietic activity apparently identical to that of antianemia factor in purified liver extract.

An in vitro study was conducted to assess the in vitro fertilization (IVF) of pig oocytes using frozen-thawed boar sperm in media supplemented with Cyanocobalamin. The results of this study suggest that there are positive effects of 0.5 µm Cyanocobalamin supplementation during the incubation of frozen-thawed boar semen on early development of IVF derived pig embryos.

The antinociceptive effect of Cyanocobalamin (Vit B12) has been reported in animal models and human studies. The dependence and tolerance were induced in male mice using subcutaneous morphine injections, 3 times a day (50, 50 and 75 mg/kg/day) for 3 days. Mice also received Vit B12 (100, 250 and 500 µg/kg), clonidine, memantine and saline intraperitoneally before morphine administration. It is concluded that co-administration of Vit B12 and morphine could reduce tolerance to analgesic effect of morphine chronic administration and also reduce its withdrawal symptoms.

In another study, the anti-nociceptive and anti-inflammatory effects of Cyanocobalamin (Vit B12) against acute and chronic pain and inflammation were evaluated in mice.

In another study, Cyanocobalamin posses marked effect on behavior as well as it also possess memory enhancing effect in mice.

Another study was designed on animal models to explore the effects on different parameters. For this the animals were given chronic dosing for 6-8 weeks during and after which the parameters were observed to determine their effects. The lymphocytes and the monocytes counts were increased significantly by intellan, while Cyanocobalamin increases RBC counts, platelet counts, monocyte counts, hematocrit etc significantly. The SGPT, SGOT were found increased in both of these drugs.

Absorbed vitamin B12 is transported via specific B12 binding proteins, transcobalamin I and II to the various tissues. The liver is the main organ for vitamin B12 storage.

Gastrointestinal absorption of vitamin B_{12} depends on the presence of sufficient intrinsic factor and calcium ions.

Cyanocobalamin is excreted mainly via the kidneys in dogs. For Cyanocobalamin, the half-lives were 0.3 to 0.5h in rats, 0.8 to 1h in dogs and 9.3 ± 3.2 hours in humans.

There have been no negative effects reported for acute toxicity, chronic toxicity or reproduction of Cyanocobalamin.

Cyanocobalamin has a low acute toxicity. The main target organs of toxicity in repeat-dose studies were the liver, kidney and bone marrow, with findings suggestive of tissue overload with high quantities of Cyanocobalamin.

Convulsions, followed by cardiac or respiratory failure, have been described in mice given 1.5-3 mg/kg body weight of vitamin B12; clinical signs were not thought to be due to hypersensitivity.

No toxic effects of B12 have been encountered in human or animal at any level of oral intake [redacted]

No long-term animal studies have been performed to evaluate carcinogenic or mutagenic potential, or whether this drug affects fertility of males or females

No systematic toxicological studies have been reported for vitamin B12. There are no reports attributing carcinogenic or mutagenic or teratogenic potential to Cyanocobalamin. In one study a tumour promoting effect was reported in a rat model, but this study is not considered relevant for safety assessment in humans.

Adequate reproduction studies have not been performed in animals to determine whether this drug affects fertility in males or females, has teratogenic potential, or has other adverse effects on the fetus. This drug should be used in pregnant women only when clearly needed.

All the information as discussed in this non-clinical overview is included in the appropriate sections of the Smpc and PIL.

[redacted]

