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Veterinary Medicines and Inspections

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COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE

EUROPEAN PUBLIC MRL ASSESSMENT REPORT (EPMAR)

LECTIN EXTRACTED FROM RED KIDNEY BEANS (*Phaseolus vulgaris)*
Porcine species

INTRODUCTION

On 16 June 2008 the European Commission adopted a Regulation[1] including lectin extracted from red kidney beans (*Phaseolus vulgaris)* in Annex II of Council Regulation (EEC) No 2377/90 for porcine species (oral use only). This decision was based on the favourable opinion and the assessment report adopted by the Committee for Medicinal Products for Veterinary Use.

Lectin extracted from red kidney beans (*Phaseolus vulgaris)* is intended for use in porcine species for the reduction of weaning disorders such as prevention of diarrhoea via oral administration.

Biolek Sp Zoo submitted the application for the establishment of maximum residue limits to the European Medicines Agency (EMEA), on 4 October 2007.

Based on the data in the dossier, the Committee for Medicinal Products for Veterinary Use concluded that it was not necessary for the protection of public health to establish maximum residue limits in porcine species for the substance when used orally and therefore lectin extracted from red kidney beans should be included in Annex II of Council Regulation (EEC) 2377/90, in accordance with Article 3. Subsequently the Commission recommended on 21 April 2008 the inclusion of the substance in Annex II for porcine species (oral use only). This recommendation was confirmed on 13 May 2008 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 16 June 2008.

EXPLANATORY NOTE

The European Public MRL Assessment Report is a public document giving the overview of the assessment carried out by the Committee for Medicinal Products for Veterinary Use (CVMP) of an application submitted for the establishment of maximum residue limits (MRLs). The document is based on the CVMP assessment report of the application from which confidential information has been deleted.

KEY WORDS: lectin, red kidney beans, *Phaseolus vulgaris*, porcine

[1] Commission Regulation (EC) No 542/2008, O.J. L 157, of 17.06.2008

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SUMMARY OF THE SCIENTIFIC DISCUSSION FOR THE ESTABLISHMENT OF MRLs

Substance name:	Lectin extracted from red kidney beans (*Phaseolus vulgaris)*
Procedure number:	EU/07/161/BLK
Applicant:	BIOLEK Sp. z o.o.
Target species:	Porcine
Intended therapeutic indication:	Reduction of weaning disorders such as prevention of diarrhoea
Route (s) of administration:	Oral

1. Introduction

Lectins are proteins of a non-immunoglobulin nature that bind carbohydrates in a reversible and specific manner, and often have haemagglutinating properties. Lectins are a ubiquitous plant constituent, but the *Leguminosae* and seeds are especially rich in these sugar binding proteins.

The lectin extracted from red kidney beans (*Phaseolus vulgaris*) is a mixture of phytohaemagglutinin (PHA) isoforms. The protein is composed of two molecular species, a leucoagglutinin (PHA-L) and an erythroagglutinin (PHA-E). The lectin exists as five isolectins: PHA-E_4, PHA-E_3L, PHA-E_2L_2, PHA-EL_3, PHA-L_4; each being a tetramer held together by noncovalent force. The isolectins have similar amino acid compositions that differ only in threonine, lysine, and arginine. Despite the differences in individual basic amino acids, the total basic amino acid content is identical for each of isolectins. A distinguishing feature of the amino acid composition is the total lack of sulfur-containing amino acids. The carbohydrate compositions of the isolectins are also similar; each isolectin contains about 4% of mannose and 2.2% of N-acetyl-o-glucosamine.

Phaseolus vulgaris varieties differ greatly in their composition and content of lectin. The lectin is concentrated in the albumin fraction with no detectable lectin activity being found in the main protein (globulin) fraction. Lectin can be obtained from plant material in a multi-step or one-step process of acid extraction of red kidney bean seeds followed by dialysis and concentration processes.

The relative contribution of isolectins may vary according to the variant of the kidney bean, soil, climate and other factors. Consequently, for determining biological activity, the haemagglutination activity (expressed as Haemagglutination Activity Units - HAU) of lectin is more important than the PHA content.

In veterinary medicine, lectin extracted from red kidney beans (*Phaseolus vulgaris)* is intended for use in products for single oral administration to suckling piglets between day 10 and 15 of their life. The main indication is the reduction of weaning disorders such as prevention of diarrhoea. The recommended dose is 320 HAU/piglet of less than 2kg or 640 HAU/piglet weighing 2 to 5 kg.

2. Safety assessment

2.1 *Overview of pharmacological properties*

Pharcodynamic properties including mode of action

General effects in humans of a single exposure to large doses of kidney bean lectin resulting from ingestion of raw kidney beans or oral administration of purified kidney bean lectin are as follows-

nausea, bloating, vomiting and diarrhoea. In experimental animals fed on diets containing plant lectins the evident symptoms are loss of appetite, decreased body weight and eventually death. The severity of symptoms is dose-dependent.

The general pharmacodynamic effects of red kidney beans, when administered by the oral route, are related to the effects on the gastrointestinal mucosa, where a maturation effect and mitotic effect are observed after treatment. The interaction of PHA with the gut mucosa seems to occur by binding of PHA to the complex glycosyl side chains occurring on the surface of the mucosa brush border. The mitogenic effects of isolectins L_4 and E_4 were demonstrated by a high affinity of these lectins to the lymphocyte surface. Also the maturation effects of PHA can be related to an increase of polyamine concentrations in the intestinal tissues, indicating that the maturation effects would be the result of multiple factors. Finally some effects on insulin, thyroxine, glucagon, cholesterol, corticosterone, and testosterone levels could be observed after long exposures indicating some modifications in the metabolism of the treated animals. The best known effect is the stimulation of cholecystokinin (CCK) release from the CCK-producing cells located in the intestinal mucosa, and trophic effect of CCK on the exocrine pancreas in rats and pigs.

Addition of 42 mg pure PHA per rat per day for 10 days compared to a control lactalbumin diet led to a reduction of wet weight of the stomach from 666±17 mg to 588±42 mg, an increase of the wet weight of the small intestine from 4308±78 mg to 6255±294 mg and cecum from 470±5 mg to 559±21 mg in the stomach, total protein, RNA, and DNA contents were significantly reduced in PHA-treated rats as compared to controls. This reduction was, however, proportional to the reduction of the total body weight. In contrast, in the small intestine the total protein, RNA, and DNA contents were significantly increased in PHA-treated rats as compared to controls indicating hyperplastic growth. In the cecum the total protein and RNA, but not DNA, contents were also significantly increased in PHA treated rats. Lectin was bound to the surface epithelium and the parietal cells region in the stomach as demonstrated using polyclonal PHA-antibody detected by PAP staining. The gastric emptying was slower in PHA-treated rats as compared to controls: after killing the animals 2 hours after PHA treatment, the stomach still contained significantly larger amounts of food than that in control rats. The stomach content pH was increased in PHA-treated rats to pH 5.6-6.2 as compared to pH 2.5-3.5 in control rats. Binding to the stomach surface disappeared within three days after the last administration of PHA but was still present in the parietal cells. In the small intestine, PHA was bound to the epithelium of the intestinal villi and produced a growth factor-like effect. After receiving PHA at daily doses of 0.65, 1.3, 2.6, 5.2, 10.5, 15, 21, 30, or 42 mg/rat (i.e., respectively, 8.12, 16.25, 32.5, 65, 131.25, 187.5, 262.5, 375, or 525 mg/kg body weight at the beginning of the study) for 10 days, the length and thickness of the small intestine of the rats were increased in a dose-dependent manner at doses between 0.65 and 21 mg/rat per day. Doses higher than 21 mg/rat per day did not produce further growth promoting effects. The growth effects became significant after 3 days of treatment, and partially reversed within 3 days after removal of PHA from the diet. Lectin bound to the surface of cecum and colon. The increases in the weight, protein, RNA, and polyamine contents indicated an increase of metabolic activity in the large intestine tissues. The growth effects were, however, not observed within the first three days of PHA treatment. Within the range of PHA doses studied (0.65 to 42 mg/rat for 10 days), the doses lower than 10.5 mg per rat per day were without effect on the large intestine tissues and doses above 10.5 mg per rat per day did not produce effects in a dose-dependent manner. The effects in the large intestine were reversed after the PHA was removed from the diet. In the treated animals an increase of wet weight of the pancreas from 442±22 mg to 624±60 mg, and reduction of the liver wet weight from 3554±297 mg to 3047±183 mg was observed compared to animals on control diet. In the pancreas the total protein and RNA, but not DNA, contents were significantly increased in PHA treated rats as compared to controls suggesting growth by hypertrophy. Microscopically, an increased size of pancreatic acini was observed. The effect on the pancreas was in linear relation to both the duration of administration (10 day study) and the dose of PHA. Daily doses lower than 7 mg PHA per rat per day were not effective, while a maximal response was achieved with 21 mg of PHA per rat per day. The changes induced in the liver did not follow similar patterns to those observed in the GI tissues. Binding studies using polyclonal PHA-antibody detected by PAP did not detect PHA in the extragastroinetestinal organs (pancreas, spleen, liver, kidneys and thymus) following oral or intragastric administration of PHA.

In a study in which PHA, purified by affinity chromatography PHA, was administered into the diet of young rats weighing approximately 80 g (42 mg per rat per day) for 10 days, administration of PHA increased the weight of the small intestine and pancreas as well as elevated the concentration of plasma CCK as compared to control lactalbumine diet. The dose related release of endogenous CCK by PHA was also confirmed in the dispersed intestinal mucosal cells *in vitro*. Block of mucosal CCK_1 receptors by MK 329, a selective CCK_1 receptor inhibitor, did not affect the weight of the small intestine, indicating that the growth effect of PHA on the small intestine is direct (i.e. does not depend on endogenous CCK release). In contrast, the growth-promoting effect on the pancreas was blocked by a CCK_1 receptor antagonist by 72%, indicating a CCK-dependent mechanism. The suggested possible mechanism could be lectin binding to the glycosyl side chains of the mucosa brush border.

In a study in which rats were treated with three consecutive single intragastric doses of 50 mg PHA/kg bw, a significant reduction in body weight was observed. The effect was reversed within 3 days after the last administration. A significant increase in stomach weight and small intestine weight and diameter were observed 1 day and 3 days after treatment compared to controls. The total number of crypt cells and the number of proliferating cell nuclear antigen positive ($PCNA^+$) stained cells were increased in the PHA-treated neonatal rats indicating enhanced stem cell mitotic activity. Importantly, this effect was significant one day, but not 3 days, after the PHA treatment, which indicates that the effect was reversible. No damage to the epithelial cells was observed. In suckling rats, enterocytes of the lower half of the distal small intestine contain large supranuclear lysosomal vacuoles (fetal-type enterocytes) which normally disappear before weaning. In the neonatal rats treated with PHA on day 14, 15 and 16, the number of vacuolated enterocytes on day 17 and 19 was significantly reduced indicating increased speed of intestinal mucosa maturation and this effect was not reversible. Enhanced maturation of the gut mucosa in PHA-treated rats was also confirmed by a significant strong reduction of intestinal absorption of marker molecules (rat IgG, bovine IgG, and BSA) and change in brush border enzyme (disaccharidase, lactase, maltase, and sucrase) activity pattern as compared to their age-matched controls. The changes related to mucosa maturation were not reversible. The treatment resulted in significant increase of pancreas weight, total protein content, and trypsin activity as compared to age-matched controls. These changes were not reversible, suggesting the enhancement of the maturation process in rat neonates.

In a study where single doses of 5 or 50 mg/kg bw of purified PHA were administered by either the oral or subcutaneous routes it was observed that enteral exposure to PHA was necessary to induce the precocious intestinal maturation effects, since the parenteral (subcutaneous) administration of an identical dose of PHA did not affect the development of the small intestine morphology and function. The lack of observable effects on the pancreas after subcutaneous administration indicate that the growth promoting effect is driven via an indirect mechanism from the gut lumen. Binding studies using polyclonal PHA-antibody detected by PAP staining did not detect PHA in the extragastroinetestinal organs (pancreas, spleen, liver, kidneys, thymus) following oral administration of PHA.

In a study in which rats were treated with 22 mg of pure PHA in food for 7 days, no differences were detected in weight gain of control and PHA treated animals. The length and size of the small intestine as well as the wet weight of the intestinal mucosa were significantly increased in treated rats. PHA binding to the intestinal mucosa surface was observed. The mucosa total protein and DNA contents were significantly increased, but the sucrase specific activity was reduced in PHA-treated rats. The length of villi was reduced, and the crypt depth increased as compared to controls. The mitotic index was increased indicating enhanced cell renewal, the increase in goblet cell number and intraepithelial lymphocyte number as compared to control was also evident in PHA-treated rats. The increase in crypt stem cell proliferation activity was confirmed by a 2-fold enlargement of [^{3}H]thymidine uptake into crypt cells. These effects were reversed within 4 days after removal of PHA from the diet.

After three intragastric administrations of 400 mg/kg bw of red kidney albumin, representing 100 mg/kg bw of pure PHA, to suckling pigs a significant reduction of villi length and a significant increase of crypt depth, mitotic index, and cell apoptosis was observed in the treated animals compared to controls. Functional changes which suggest increased speed of intestinal mucosa maturation, such as enhanced disappearance of vacuolated enterocytes, kinetic changes in brush

border disaccharidase activities and reduction in intestinal tissue permeability for marker molecules, were observed. At postmortem examination no obvious differences in the total length and weight of the different small intestinal regions were found between the two groups, except that the weight of the distal jejunum was lower in lectin treated pigs. No differences in pancreas, liver, spleen, or adrenal weights were found between the two groups. Oral administration of lectin preparation (100 mg PHA/kg bw) resulted in a tendency towards higher body weight gain, improved feed conversion ratio and a marked reduction of diarrhoea index as compared to controls. In the treated animals a significant elevation of plasma CCK, and enlargement of the size of pancreatic acini was observed as compared to controls, though there were no differences in the total protein content, trypsin and amylase activities in pancreas tissue homogenates. At postmortem examination, no differences in the pancreas and liver weights were found between the two groups: control and PHA-treated. There were no signs of epithelial damage following enteral exposure to PHA. Three intragastric administrations of 400 mg red kidney bean albumin/kg bw (i.e., about 100 mg PHA/kg bw) to neonatal suckling piglets at 10, 11 and 12 days of life resulted in less body weight gain as compared to controls. Six of the 11 lectin-treated and none of the 16 control pigs showed diarrhoea symptoms during the treatment; for lectin-treated pigs the diarrhoea stopped within 24 hours in every case.

Pharmacokinetic properties (mainly in laboratory animals)

After administration of ^{125}I-labelled lectins to rats, 5 to 10% of the orally administered dose is systemically available. One hour after administration of the labelled PHA 16.44, 14.15, 0.34 percent of the dose was recovered as E3L lectin in small intestine contents, small intestine tissue and caecum and colon tissues respectively. At this time 0.10, 0.19, 0.65, 0.11, 0.07 and 0.04 percent was recovered as E3L lectin in blood cells, plasma, liver, kidney, pancreas and spleen respectively. The percentages of L4 lectin recovered in small intestine contents, small intestine tissue, caecum and colon tissues, blood cells and plasma one hour after lectin administration were respectively 7.10, 8.70, 0.25, 0.07 and 0.06. No L4 lectin was recovered in liver, kidney, pancreas and spleen one hour after administration of labelled lectins. Three hours after administration of labelled PHA 16.00, 8.70 and 0.64 percent of the dose was recovered as E3L lectin in small intestine contents, small intestine tissue and caecum and colon tissues respectively. At this time 0.20, 0.57, 0.61, 0.16, 0.04 and 0.01 percent were recovered as E3L lectin in blood cells, plasma, liver, kidney, pancreas and spleen respectively. The percentages of L4 lectin recovered in small intestine contents, small intestine tissue, caecum and colon tissues, blood cells and plasma three hours after administration of lectin were respectively 3.30, 4.48, 0.54, 0.037 and 0.43. No L4 lectin was recovered in liver, kidney, pancreas and spleen three hours after administration of labelled lectins.

Lectins from red kidney beans, purified by affinity chromatography were administered to 14 day old suckling rat pups either via a gastric tube (enteral) or by subcutaneous injection (parenteral). The dose of PHA for enteral and parenteral administration was either 0.05 or 0.005 mg/g body weight. The administrations were repeated on days 15, 16 and 17 of life. Twelve hours after treatment the rats were killed, and tissue samples (proximal small intestine, glandular stomach, pancreas, liver, spleen, thymus, kidneys) were prepared for histology analyses. The tissue binding of PHA was evaluated by immunohistochemistry using rabbit anti-*Phaseolus vulgaris* agglutinin as the primary antibody. Enterally administered PHA could be detected along the epithelial lining of the stomach and the small intestine but not in the other organs studied. Parenterally administered PHA could be detected in the pancreas, spleen, liver, kidneys and thymus but not in the epithelial lining of the stomach or the small intestine. This study clearly indicates that an oral dose of lectin given enterally to rat neonates does not cross the epithelial barrier in the gastrointestinal tract and is not absorbed.

After oral route administration of pure PHA (50 mg/kg bw) to suckling rats binding studies demonstrated that PHA bound to the brush border of enterocytes along the entire length of the villi but did not bind in the crypt region. Intense PHA intestinal binding was found within both 1 hour and 3 to 6 hours after administration, and it was reduced but still visible 12, 24, and 72 h after administration. Twelve hours after exposure, stained PHA granules were observed in the enterocytes in the proximal small intestine, and after 24 hours, in the distal small intestine. Seventy two hours after administration PHA staining was observed also on the basolateral enterocyte membrane and in the lamina propria.

In contrast to the small intestine, no PHA binding was detected in the rat pancreas, liver, thymus, or spleen at 1 to 72 hours after the PHA treatment.

2.2 *Calculation of pharmacological ADI*

A pharmacological ADI could not be established.

2.3 *Overview of toxicology*

Acute toxicity and tolerance in target species

No single dose toxicity data were available and an acute oral LD50 value has not been reported. In humans, the consumption of raw red kidney beans may lead to acute gastroenteritis, which may be severe. The deleterious effects of kidney bean lectin-containing food in humans is ascribed to the presence of undestroyed PHA by inappropriate cooking. The adverse effects generally involve nauseas, vomiting and diarrhoea.

Repeated dose toxicity

No repeated dose studies were conducted. Data from the published literature show that the target organs of kidney bean lectins seem to be the digestive tract, in particular the small intestine, and the pancreas.

Short-term (10 day) dietary exposure to purified kidney bean lectin has been shown to impair the growth of rats, induce enlargement of the small intestine, cause damage to the epithelium of the small intestine and stimulate hypertrophy and hyperplasia in the pancreas. In addition, at high dietary concentrations kidney bean lectins induce rapid depletion of the body muscle, lipid and glycogen. The highest PHA dose that does not produce significant growth effects in the rat small intestine and pancreas is 5.2 mg/rat/day (i.e., 65 mg/kg bw per day). In contrast to the damaging effects of PHA at high levels in the diet, at lower daily doses (0.01 to 0.2 g/kg bw), loss of body weight and skeletal muscle atrophy are slight. However, PHA can still exert its modulating effect on plasma insulin levels and lipid catabolism.

In a 28-day toxicity study in rats using pure recombinant PHA-E (*Phaseolus vulgaris*) lectin, a dose level of 0.08% PHA-E lectin (corresponding to a daily intake of approximately 55 and 70 mg PHA-E lectin/kg body weight for male and female rats, respectively) was reported to be the LOAEL based on the increased weight of the small intestine.

In feeding trials carried out in rats to asses the effects of long term (700 day) consumption of diets containing low and high levels of raw kidney beans on body weight, composition and organ weights, the diets reduced feed conversion efficiency and growth rates during the initial 250 days. However, after 250 days the weight gain by rats given legume-based diets was similar to those of controls given the same daily feed intake. Long term consumption of diets containing low levels of kidney beans significantly altered the body composition of rats. The levels of lipid in the body were significantly reduced. As a result, carcasses of these rats contained a higher proportion of muscle/protein than did controls. Small intestine relative weights were increased by short and long term consumption of the kidney bean based diet. However, the increase in relative pancreatic weights observed at 30 days did not persist long term. Long term consumption of the kidney bean based diets by rats resulted in a significant increase in the relative weights of the caecum and colon.

Tolerance in target species

No information was provided.

Effects on reproduction including developmental effects

No information was provided on effects on reproduction including developmental effects. From the available information and considering particularly that red kidney bean lectin is a component of human and animal diets - therefore the gut of humans and animals is continuously exposed to exogenous dietary lectins - and that pharmacokinetic studies indicate low absorption, it is considered that the risk of reproduction/developmental toxicity from residues of lectin extracted from red kidney bean (*Phaseolus vulgaris*) is not of concern.

Mutagenicity

No mutagenicity assays have been conducted. PHA from red kidney beans is used in medical research. As a substance with cytogenetic activity, it is used for the stimulation of cell proliferation in lymphocyte cell culture. It is also included in test systems that use human or other mammalian peripheral blood lymphocytes to screen for possible mammalian mutagens and carcinogens. These tests include the sister chromatid exchange test (SCE), *in vitro* Mammalian Chromosome Aberration Test (OECD TG 473) and the in vitro mammalian micronucleus assay. In these test systems the substance was not shown to be mutagenic. It is considered that the risk of mutagenicity from residues of lectin extracted from red kidney bean (*Phaseolus vulgaris*) is not of concern.

Carcinogenicity

No carcinogenicity studies for lectin extracted from *Phaseolus vulgaris* were provided. According to published studies, the development of tumours has been markedly diminished by introducing PHA (0.45 to 3.5 mg/g diet) into the diet of mice. The growth of a subcutaneous MPC-11 plasmacytoma tumour in Balb/c mice was similarly affected. Authors of the review suggested that a competition between the gut epithelium undergoing hyperplasia and the developing tumour may occur for polyamines and other nutrients from a common body pool and this may be an important factor limiting tumour growth following feeding with a PHA-containing diet. In a subsequent paper the authors found that PHA used as a dietary component (1.75, 3.5 or 7.0 mg PHA/g diet) may reduce the growth of an established non-Hodgkin lymphoma tumour in NMRI mice in a dose-dependent manner.

From the available information and considering particularly that red kidney bean lectin is a component of human and animal diet - therefore the gut of humans and animals is continuously exposed to exogenous dietary lectins - and that pharmacokinetic studies indicate low absorption, it was considered that the risk of carcinogenicity from residues of lectin extracted from red kidney bean (*Phaseolus vulgaris*) is not of concern.

Studies of other effects including immunotoxicity and neurotoxicity

A review of published literature on PHA interference with the local (gut) immune system shows that orally presented lectin from red kidney beans (PHA) is a very potent immunogen and produces an exclusive and powerful humoral, IgG-type, anti-lectin response. It has since been shown that all other lectins are similar regardless of whether they have been presented parenterally or orally. In contrast, all non-lectin proteins and lectins that have been denatured by chemical or physical methods, are only immunogenic when administered parenterally. When given orally they elicit a minimal or no antibody response.

In addition to local hypersensitivity reactions to dietary PHA, both mice and rats develop systemic, immediate-type (Type-1) hypersensitivity due to the production of systemic anti-lectin IgE. Mice are sensitized to dietary PHA and this can be demonstrated by the booster effects in IgE production after the intraperitoneal injection of PHA with $Al(OH)_3$ as adjuvant. Similar, though less extensive booster effects have also been observed after the oral re-administration of PHA 10 days after the initial exposure. Although the extent and the nature of systemic allergic responses and their contribution to the antinutritional effects of PHA are not known, as some lectins, such as PHA or jacalin, suppress the IgE response to other simultaneously applied antigens, consumption or therapeutic application of lectins may be used to alleviate allergic responses in sensitive people.

2.4 *Calculation of the toxicological ADI*

NOELs from repeated dose and developmental toxicology studies using lectin extracted from red kidney beans were not available and an ADI for the lectin extracted from the red kidney beans could not be established.

2.5 *Overview of microbiological effects*

Lectin does not have antimicrobial activity itself but induces changes in the intestinal microbial flora that contributes to the malabsorption of nutrients in the small intestine of PHA-fed animals. However as residues are only expected at very low concentrations a specific assessment of microbiological consumer hazard of potential residues is not considered necessary.

2.6 Calculation of microbiological ADI

Not required due to the lack of microbiological activity of the substance.

2.7. *Overview of evaluation by other international committees*

Not applicable

2.6. *Overall conclusions on the ADI*

An overall ADI could not be established.

3. Residues assessment

3.1 *Pharmacokinetics in target species*

No specific pharmacokinetic studies have been performed with lectin extracted from red kidney beans (*Phaseolus vulgaris*) in target animals.

3.2 *Residue depletion studies*

Residue depletion studies for lectin extracted from red kidney beans (*Phaseolus vulgaris*) were not provided. Limited pharmacokinetic data on PHA in rats indicate relatively low absorption rates of 5 to 10% of the orally administered dose of ^{125}I-labelled lectin.

Lectins are a ubiquitous plant constituent, and members of the *Leguminosae* seeds are rich in these sugar binding proteins. Lectins are therefore constituents of daily feed and food intake. Human dietary exposure from plant sources can be expected to far exceed the potential exposure to lectin extracted from red kidney beans via residues in food-producing animals. The highest estimated dietary short term intake of beans without pods is about 5 g/kg bw (300g/person) in adults (EFSA, Food consumption data). Taking into account that 1 g of red beans contains 200 to 400 HAU, the maximal daily amount ingested with a single meal could be around 120 000 HAU. This is several orders of magnitude higher than the total dose in piglets (320 to 640 HAU/piglet), which provides a significant margin of safety.

3.3 *Selection of marker residue and target tissues*

See 3.4.

3.4 *Elaboration of MRLs*

In view of the fact that the substance is a common plant component and a normal component of the human diet, it is not considered necessary for the protection of public health to establish MRLs.

3.5 *Calculation of theoretical daily intake of residues*

Not applicable

3.6 *Analytical method for monitoring of residues*

No analytical method for monitoring of residues was provided, however as the establishment of MRL values is not considered necessary for this substance, an analytical method is not required.

3.7 *Considerations on possible extension of MRLs if applicable.*

Considerations were given to the possible extension of the recommendation for porcine species to other species. However, taking into account the limited set of data available, a further extension of the recommendation is not considered possible.

4. Conclusions and recommendation

Having considered the criteria laid down by the Committee for Medicinal Products for Veterinary Use for the inclusion of substances in Annex II of Council Regulation (EEC) No 2377/90 and in particular that:

- lectins are a common plant component,
- lectin extracted from red kidney beans (*Phaseolus vulgaris)* is a normal component of the diet in humans,
- lectin extracted from red kidney beans (*Phaseolus vulgaris)* is expected to be used in a small number of individual animals only, for infrequent or non-regular treatment;

the Committee for Medicinal Products for Veterinary Use (CVMP) concludes that there is no need to establish MRLs for lectin extracted from red kidney beans (*Phaseolus vulgaris*) and recommends the inclusion of the substance in Annex II of Council Regulation (EEC) 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
Lectin extracted from red kidney beans (*Phaseolus vulgaris*)	Porcine	For oral use only

Glossary

ADI – Acceptable daily intake
MRL – Maximum residue limit
NOEL – Non-observed effect level

Background information on the procedure

Submission of the dossier:	4 October 2007
Steps taken for assessment of the substance:	
Application validated:	18 October 2007
Clock started:	19 October 2007
CVMP opinion adopted:	16 January 2008