COLLEGE TER BEOORDELING VAN GENEESMIDDELEN

Public Assessment Report

Scientific discussion

Cleenema 180.8 mg/ml –/79.9 mg/ml, rectal solution (sodium dihydrogen phosphate dihydrate/disodium phosphate dodecahydrate)

(NL/H/5285/001/DC)

Date: 31 May 2022

This module reflects the scientific discussion for the approval of Cleenema. The procedure was finalised at 10 December 2021. For information on changes after this date please refer to the 'steps taken after finalisation' at the end of this PAR.

List of abbreviations

ASMF	Active Substance Master File
BP	Bowel preparation
cGMP	cyclic guanosine monophosphate
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
ECG	electrocardiogram
EDMF	European Drug Master File
EDQM	European Directorate for the Quality of Medicines
EEA	European Economic Area
ERA	Environmental Risk Assessment
GFR	glomerular filtration rate
ICH	International Conference of Harmonisation
K_m	Michaelis constant
MAH	Marketing Authorisation Holder
NaP	Sodium phosphate
PEG	Polyethylene glycol
Ph.Eur.	European Pharmacopoeia
PL	Package Leaflet
RH	Relative Humidity
RMP	Risk Management Plan
SmPC	Summary of Product Characteristics
TmP	Tubular reabsorption capacity
TNBS	2,4,6-trinitrobenzene sulfonic acid
TSE	Transmissible Spongiform Encephalopathy
V_{max}	Maximal velocity

I. INTRODUCTION

Based on the review of the quality, safety and efficacy data, the Member States have granted a marketing authorisation for Cleenema 180.8 mg/ml –/79.9 mg/ml, rectal solution from Casen Recordati S.L.

The product is indicated in adults for the cleansing of the rectum, the sigmoideum and the lower part of the colon descendens:

- in case of occasional constipation;
- if needed, for the preparation of medical and diagnostic procedures.

A comprehensive description of the indications and posology is given in the SmPC.

The proposed indications for Cleenema are in line with those of medicinal products containing sodium dihydrogen phosphate dihydrate and disodium phosphate dodecahydrate as active substances which are authorized for marketing within the European Union for more than 10 years. Current marketing authorizations, available evidence in medical literature, and sales data of sodium phosphate enemas within the European Union in the last 10 years support the well-established use of sodium phosphate for proposed indications.

The concerned member states (CMS) involved in this procedure were Austria, Czech Republic, Hungary and Slovakia.

The marketing authorisation has been granted pursuant to Article 10a of Directive 2001/83/EC, a so called bibliographic application based on the well-established medicinal use of medicinal products containing sodium dihydrogen phosphate dihydrate and disodium phosphate dodecahydrate as active substances. This type of application does not require submission of the results of pre-clinical tests or clinical trials if it can be demonstrated that the active substance of the medicinal product has been in well-established medicinal use within the community for at least ten years, with recognised efficacy and an acceptable level of safety. These requirements were met, therefore the marketing authorisation was granted.

II. QUALITY ASPECTS

II.1 Introduction

Cleenema is a clear, colourless, odourless solution, free from precipitate and turbidity.

Cleenema contains as active substances 180.8 mg of sodium dihydrogen phosphate dihydrate (equivalent to 139.1 mg of sodium dihydrogen phosphate anhydrous) and 79.9 mg of disodium phosphate dodecahydrate (equivalent to 31.7 mg of disodium phosphate anhydrous) per ml.

The rectal solution (enema) is packed in LDPE squeeze translucent bottles.

The excipients are disodium edetate, benzalkonium chloride, water and white soft paraffin (nozzle lubricant).

II.2 Drug Substance

Phosphoric acid
The active substance is phosphoric acid diluted, an established active substance described in the European Pharmacopoeia (Ph.Eur.). Phosphoric acid is a clear, colourless, syrupy liquid. The active substance is a 75% solution in water and is miscible in both water and ethanol. Polymorphism and/or stereochemistry are not relevant.

Manufacturing process
The substance concerns an atypical active substance of inorganic origin. Thermal phosphoric acid is produced by combustion. The process description is general but sufficient considering the type of substance. Solvents, reagents and catalysts are not applicable. The active substance has been adequately characterized.

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. with additional requirements for potential elemental impurities. Batch analytical data demonstrating compliance with this specification have been provided for three batches.

Stability of drug substance
The active substance is fully tested to ensure compliance with its specification immediately prior to its use in manufacture of the product.

Sodium hydroxide
The active substance is sodium hydroxide, an established active substance described in the European Pharmacopoeia (Ph.Eur.). Sodium hydroxide is a white, crystalline mass. The active substance is a 50% solution in water and is miscible in both water and alcohol. Polymorphism and/or stereochemistry are not relevant.

Manufacturing process
Sodium hydroxide solution is produced by electrolysis. No solvents or catalysts are used in the process. Elemental impurities are the only possible impurities. Acceptable control strategies have been adopted for the starting material.

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. Furthermore, density is determined according to Ph. Eur. method. Batch analytical data demonstrating compliance with this specification have been provided for three batches from each supplier.

Stability of drug substance
The active substance is fully tested to ensure compliance with its specification immediately prior to its use in manufacture of the product.

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 development of the product has been described, the choice of excipients is justified and their functions explained. The need to include benzalkonium chloride and its concentration are adequately justified taking into account the Ph.Eur. requirements for this type of rectal dosage form. The provided literature is acceptable to support the application as for each indication relevant literature using the product at issue has been submitted.

Manufacturing process
The manufacturing process has been validated according to relevant European/ICH guidelines. The manufacturing process is straightforward and includes dissolution of the substances which react in situ to form the intended active substances. Process validation data on the product have been presented for three batches in accordance with the relevant European guidelines.

Control of excipients
The excipients comply with Ph. Eur. Requirements. 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 sodium and phosphates, pH, assay disodium phosphate dodecahydrate; assay sodium dihydrogen phosphate dihydrate, specific gravity, benzalkonium chloride, total viable count, mould and yeast, preservative efficacy testing, specific gravity and uniformity of dosage units. Limits in the specification have been justified and are considered appropriate for adequate quality control of the product. The potential presence of elemental impurities and nitrosamines has sufficiently been discussed.

Satisfactory validation data for the analytical methods have been provided.

Batch analytical data three production scaled batches from the proposed production site has been provided, demonstrating compliance with the specification.

Stability of drug product
Stability data on the product have been provided for three production scaled batches stored at 25°C/60% RH (36 months), 30°/65% RH (12 months) and 40°C/75% RH (six months) in accordance with applicable European guidelines demonstrating the stability of the product for

36 months. The batches were stored in the proposed container. Photostability studies demonstrated that the drug product is sensitive to light. On basis of the data submitted, a shelf life was granted of 36 months. The medicinal product does not require any special temperature storage conditions. The bottle should be kept in the outer carton for protection from light.

Specific measures concerning the prevention of the transmission of animal spongiform encephalopathies
There are no substances of ruminant animal origin present in the product nor have any been used in the manufacturing of this product, so a theoretical risk of transmitting TSE can be excluded.

II.4 Discussion on chemical, pharmaceutical and biological aspects

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

III. NON-CLINICAL ASPECTS

III.1 Pharmacology

III.1.1 Primary pharmacodynamics

Ordinarily, defecation is initiated by defecation reflexes. One of these reflexes is an intrinsic reflex mediated by the local enteric nervous system in the rectal wall. This can be described as follows: When faeces enter the rectum, distention of the rectal wall initiates afferent signals that spread through the myenteric plexus to initiate peristaltic waves in the descending colon, sigmoid, and rectum, forcing faeces toward the anus. As the peristaltic wave approaches the anus, the internal anal sphincter is relaxed by inhibitory signals from the myenteric plexus; if the external anal sphincter is also consciously, voluntarily relaxed at the same time, defecation occurs. The intrinsic myenteric defecation reflex functioning by itself normally is relatively weak. To be effective in causing defecation, it usually must be fortified by another type of defecation reflex, a parasympathetic defecation reflex that involves the sacral segments of the spinal cord. When the nerve endings in the rectum are stimulated, signals are transmitted first into the spinal cord and then reflexly back to the descending colon, sigmoid, rectum, and anus by way of parasympathetic nerve fibres in the pelvic nerves. These parasympathetic signals greatly intensify the peristaltic waves as well as relax the internal anal sphincter, thus converting the intrinsic myenteric defecation reflex from a weak effort into a powerful process of defecation that is sometimes effective in emptying the large bowel all the way from the splenic flexure of the colon to the anus. Defecation signals entering the spinal cord initiate other effects, such as taking a deep breath, closure of the glottis, and contraction of the abdominal wall muscles to force the faecal contents of the colon downward and at the same

time cause the pelvic floor to relax downward and pull outward on the anal ring to evaginate the feces (Guyton, 2006b).

Mono and dibasic sodium phosphates are examples of saline laxatives, a group which also includes several magnesium salts and various sulphates (Jafri, 2001). These substances are each characterised by slow and incomplete absorption from the gastrointestinal tract and their presence in the large intestine is associated with retention of water in the intestinal lumen as a result of osmotic forces. Water normally accounts for 70 to 85 % of total stool weight and reflects a balance between luminal input (ingestion and secretion of water and electrolytes) and output (absorption) along the length of the gastrointestinal tract.

In humans, investigations using Fleet Phospho-soda (2 oz added to 4 oz tap water and 0.5 oz hydrogen peroxide) instilled into the rectum demonstrated that effective bowel cleansing could be achieved within minutes, the bowel movements occurring within two to five minutes of administration (Marks, 1951). Similar times for a sodium phosphate enema to induce bowel evacuation in humans (5-14 minutes) have been reported by others (Knoernschild, 1962; Riley, 1966).

III.1.2 Mechanism of action

Although cathartics, including saline laxatives, have been used for many years, the precise mode of action of the active agents in the proposed formulation is not fully understood. Their cathartic action was believed to result from osmotically mediated water retention (Jafri, 2001). Increased water content in the colonic lumen causes an increase in stool volume and distension of the gut wall and increased peristalsis due to activation of local neural reflexes. Other mechanisms may contribute to their effects (see below). The latency and intensity of the bowel emptying effects of saline laxatives in humans is related to both the individual salt and dosage administered.

Various mechanisms of action have been proposed. These include (a) relaxation of the bowel musculature, combined with a mucorrheic and hydragogue effect (Marks, 1951), (b) an osmotic effect causing increased intraluminal volume with subsequent rapid distension of the left colon (Steinberg, 1965), (c) slight distension and irritant action on the rectal mucosa (Tillery, 1966) and (d) faecal softening, stimulation of peristalsis and detergent action (Kemp, 1971). It is likely that the phosphate enema is effective, at least in part, because of its irritant action on the rectal mucosa, as evidenced by mucorrhoea or hyperaemia, together with the slight rectal distension produced directly by the enema, rather than osmotic action (Knoernchild, 1962; Riley, 1966).

The role of volume in the action of hypertonic sodium phosphate enemas in adults has been investigated following rectal instillation of 109 cc (range 97-119 cc) of an aqueous enema containing 160 mg/cc monobasic sodium phosphate, 60 mg/cc dibasic sodium phosphate and 2.5 mg/cc hydrogen peroxide (preservative) to 50 subjects (Flentie, 1957). The average retention time for the enema was four minutes (range 1-10 minutes) and the volume of fluid evacuated was 101 cc (range 34-153 cc). The action of hypertonic enemas could not be

explained on the basis of water in the lumen of the bowel in response to an osmotic gradient, as not more than 19 cc of water moved into the colon following the administration of one adult size enema. The hypertonic sodium phosphate enema did not cause any significant loss of body water and its effect did not appear to be induced by the volume administered or by fluid abstracted from the intestinal mucosa.

More recent investigations bring into question the classification of laxatives into stimulants of motility, osmotics and bulk formers or hydrophilic colloids. In the rat intestine and colon, biological factors such as nitric oxide, platelet activating factor and other endogenous mediators have been implicated in the action of some laxatives (Jafri, 2001; Izzo, 1998). Factors such as the possible involvement of platelet activating factor and nitric oxide suggest a far more complex picture for the effects of laxatives than previously thought. Platelet activating factor produces significant stimulation of colonic secretion and gastrointestinal motility. Nitric oxide may also be involved in stimulation of intestinal secretion via prostaglandin- and cyclic guanosine monophosphate (cGMP)-dependent mechanisms. In addition, nitric oxide may inhibit segmenting contractions in the colon, promoting laxation. Although most laxatives affect nearly all aspects of active and passive electrolyte transport, establishing which of these effects is the primary contributor to laxative action and how the different mediators co-operate to produce a laxative effect requires further investigation.

III.1.3 Secondary pharmacodynamics

The effects of intravenous administration of a mixture of monobasic (5.5 g/l) and dibasic (29.5 g/l) sodium phosphate (pH 7.4) has been investigated in New Zealand White rabbits (Moore, 1988). Two groups received an intravenous dose of phosphate approximating to the equivalent of either 4 or 8 g/hour in humans at an infusion rate of 50 ml/hour for one hour. A third group received 50 ml saline (vehicle control) and a fourth group was subjected to the same procedures but received no dose (sham control). Each group consisted of 10 rabbits. Blood samples were obtained before infusion and at 0.5 hour intervals from the start of infusion to four hours after completion of the infusion. Before each sampling, electrocardiogram (ECG) traces were recorded from each rabbit. As expected, the infusions resulted in a rapid rise in plasma phosphate levels accompanied by a parallel slight fall in plasma calcium levels. The decrease in plasma calcium post-infusion, although statistically significant, was not considered to be clinically significant. The plasma levels of both phosphate and calcium returned to near control levels by four hours post-infusion. The cardiovascular system, as assessed from ECG and heart rate changes, was undisturbed by treatment. Macroscopic and microscopic pathology evaluation after the last sampling revealed no acute organ damage.

Phosphate homeostasis is known to play an important role in the development of secondary hyperparathyroidism in renal failure and may directly affect parathyroid hormone secretion. Based on this information, investigations were performed to determine whether phosphate stimulated parathyroid hormone in dogs in the absence of changes in calcium (Estepa, 1999). Mongrel dogs received total doses of 0 (saline control), 1.2, 1.6 or 2.4 mmol/kg phosphate, infused intravenously over 120 minutes to groups of 10, 8, 11 and 17 dogs, respectively. To

maintain the desired serum concentrations the infusion rate of phosphate was progressively decreased during the 120 minutes. Serum calcium was clamped at normal levels by intravenous infusion of 0.077 M (pH 7.4) calcium chloride. The infusion rate of calcium was slowly increased until 70 minutes, after which the rate was slowly decreased to maintain a constant ionised calcium concentration. In a separate group of six dogs receiving 2.4 mmol/kg phosphate, serum magnesium was clamped by infusing 0.081 M magnesium sulphate (pH 7.4); the rate of magnesium infusion was decreased during the study (total magnesium dose 0.052 mmol/kg, mean 0.026 mmol/kg/h).

A similar pattern has been reported in seven healthy adult humans receiving two oral doses of 45 ml sodium phosphate (Fleet Phospho-soda oral saline laxative), separated by 12 hours, giving a total dose of 5.76 g of elemental phosphorus (DiPalma, 1996). Analyses over the initial 24 hours from the start of treatment showed a marked rise in phosphorus (mean peak 7.6 ± 0.1 mg/dl, peak range 3.6 to 12.4 mg/dl, $P < 0.001$), associated with rises in parathyroid hormone (mean peak 317 ± 19.5 pg/ml, $P < 0.001$) and urinary cAMP (mean peak 12.9 ± 2.4 mmol/l, $P = 0.02$), and falls in total calcium (mean nadir 8.4 ± 0.1 mg/dl, nadir range 8.0 to 9.8 mg/dl, $P < 0.001$) and ionised calcium (mean nadir 4.6 ± 0.1 mg/dl, nadir range 4.4 to 5.2 mg/dl, $P < 0.001$), caused by calcium phosphate salt deposition primarily in kidneys, heart, blood vessels, cornea, lungs and gastric mucosa. No other serum and urine laboratory assessments were affected and blood pressure, pulse and respiration rate remained undisturbed.

The effects of a commercial hypertonic sodium phosphate enema administered, with five minutes enforced retention, to healthy cats at the lower and upper doses recommended for animals weighing 11.35 kg or less, have been reported (Atkins, 1985). One male and four female healthy adult cats received a dose of 60 ml (18 ml/kg, range 13 to 26 ml/kg) and three males and two females received 120 ml (32 ml/kg, range 28 to 43 ml/kg) of Fleet brand veterinary enema. The anus of each cat was digitally compressed for five minutes after administration (comparable with the retention time in human patients), preventing expulsion of the contents. Blood samples were taken and rectal temperature and heart rate were recorded at intervals up to 24 hours after administration. One cat from each group was allowed to recover, the remaining cats were subjected to necropsy after the 24-hour sampling interval and macroscopic and microscopic examinations of the tissues were performed. One cat receiving a dose of 120 ml died. All treated cats showed abnormal clinical signs, generally more severe in the 120 ml group. Most cats had ataxia and somnolence, severe in some cases, whilst vomiting, bloody diarrhoea and pale mucous membranes were less frequent. Tetany was not evident. The heart rate of cats receiving 120 ml increased from 190 beats/min to 240 beats/min 30 minutes after administration. Consistent with observations in other species, the main change was a marked and dose-related hyperphosphataemia, which remained for about four hours. Hypernatraemia, hypocalcaemia and marked hyperglycaemia were also evident. In addition, both treated groups exhibited a calculate hyperosmolality and metabolic acidosis. Hyperlacticacidaemia was also evident in both groups.

III.1.4 Safety pharmacology

As peritoneal absorption can result from perforations in the colon, the absorption of sodium phosphate following intraperitoneal administration was investigated in albino rats (Lochbühler, 1995). A ready-made enema solution (Practo-Clyss, Schiwa) was used, containing 160 mg/ml monobasic sodium phosphate and 60 mg/ml dibasic sodium phosphate. Initial investigations revealed that intraperitoneal administration of 25 ml/kg bodyweight was lethal. Subsequently, doses of 10, 12, 13, 14, 15 and 20 ml/kg were administered to groups of 16, 2, 2, 2, 6 and 6 rats, respectively. Controls (6 rats) received 20 ml/kg of sorbit solution with the same osmolality as the sodium phosphate solution (2500 mosm/l). Serum sodium, potassium, calcium, and phosphate and the volume of ascites were determined. For controls, serum sodium, potassium and calcium and volume of ascites were reported. Animals receiving 10 ml/kg were sacrificed 4, 8, 12 and 24 hours after dosing. Histological examinations were performed on the liver, kidney and peritoneum.

The potential for sodium phosphate to damage the colonic mucosa of rats has been investigated (Coskun, 2001). Groups of ten male Wistar rats received a single oral (gavage) administration of either 1.5 ml/kg sodium phosphate or 66ml/kg polyethylene glycol (PEG). A further 10 rats (control group) were given water to drink. After eight hours all rats were sacrificed and tissue samples of colon above the pelvic peritoneum were taken for microscopic evaluation and determination of oxidant activity (determination of malonyldialdehyde concentration) and antioxidative activity (glutathione peroxidase and superoxide dismutase activities) in colon homogenates.

In dogs, hyperphosphataemia stimulated a delayed, transient parathyroid hormone secretion following intravenous infusion of phosphate, even when normal ionised calcium concentration was maintained (Estepa, 1999). In the dialysis patient, the magnitude of hyperphosphataemia often exceeds normal values. Therefore, any modest increase in serum phosphate in these patients might enhance parathyroid secretion. Hyperphosphataemia with elevated parathyroid hormone and urinary cAMP levels, and hypocalcaemia have been reported in humans after oral administration of sodium phosphate (DiPalma, 1996). In this publication the authors recommended that oral sodium phosphate should not be administered in patients with cardiopulmonary, renal or hepatic disease.

In the cat study reported by Atkins *et al* (Atkins, 1985), all cats receiving the lower (60 ml, 18 ml/kg) or upper (120 ml, 32 ml/kg) recommended dose of a commercial hypertonic sodium phosphate enema, plus five minutes enforced retention, showed abnormal clinical signs and/or changes in clinical chemistry values. The most marked signs were those of neurological dysfunction (depression, stupor, somnolence and ataxia). The neurological dysfunction was probably due to fluid egress from the central nervous system secondary to hypertonicity. Although both treated groups exhibited a calculate hyperosmolality and metabolic acidosis, the necropsy examinations revealed no gross or macroscopic lesions associated with administration of the enema.

III.1.5 Pharmacodynamic drug interactions

The electrolytic changes reported in animal species are similar to those observed in humans following overdoses of sodium phosphate. However, such disturbances are uncommon in otherwise healthy patients receiving the recommended rectal instillation of monobasic and dibasic sodium phosphate.

The pharmacodynamic potential to produce changes in the electrolyte balance raises the possibility of inducing adverse effects within the central nervous, cardiovascular and renal systems. Rectal sodium phosphate administration is not recommended in patients with cardiopulmonary or renal disease. It is also possible that concomitant use of diuretics and other medications that affect electrolytes could increase the potential of sodium phosphate to induce disturbances in the electrolyte balance. The possibility of peritoneal absorption in patients with inflammatory bowel disease with a high risk of laceration of the mucosa or perforation of the bowel may increase the potential for rectal instillation of sodium phosphate to induce effects on electrolytes.

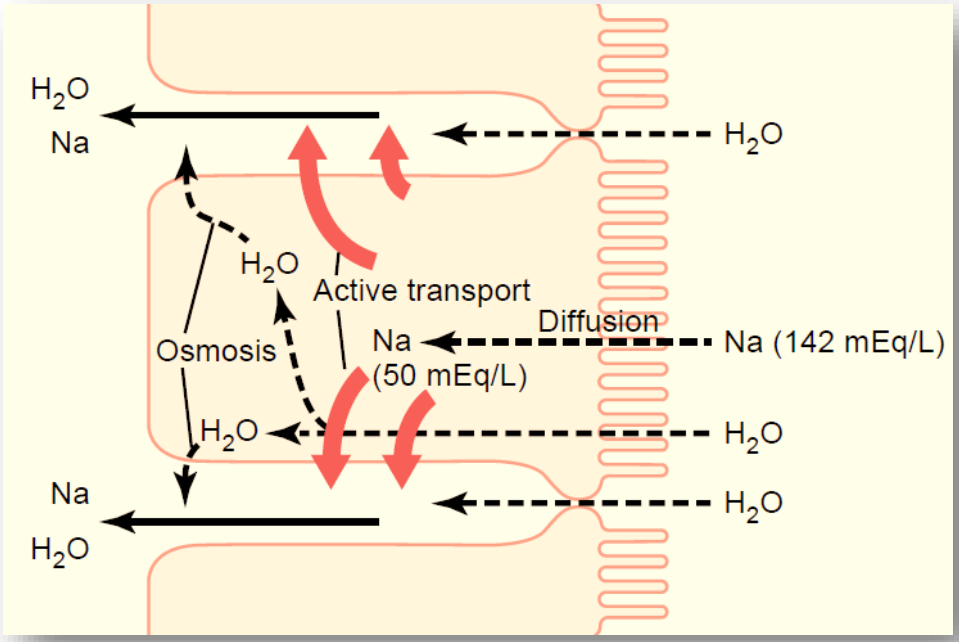
As expected with most products that have been in use for many years, the number of animal studies on monobasic or dibasic sodium phosphate is limited. However, these studies display no unexpected findings compared to those observed in humans. Based on the animal studies, the combination of monobasic and dibasic sodium phosphate in Cleenema 139,1 mg/ml – 31,7 mg/ml, when used for the indications described in the datasheet and at the recommended dosage levels in patients with no absolute or relative medical contraindications is likely to be a safe and effective product.

III.2 Pharmacokinetics

III.2.1 Absorption

The basic mechanism of sodium absorption from the intestine is shown in Figure 1. The motive power for sodium absorption is provided by active transport of sodium from inside the epithelial cells through the basal and side walls of these cells into paracellular spaces. This is demonstrated by the heavy red arrows in Figure 1. This active transport obeys the usual laws of active transport: it requires energy, and the energy process is catalysed by appropriate adenosine triphosphatase enzymes in the cell membrane. Part of the sodium is absorbed along with chloride ions; in fact, the negatively charged chloride ions are mainly passively “dragged” by the positive electrical charges of the sodium ions. Active transport of sodium through the basolateral membranes of the cell reduces the sodium concentration inside the cell to a low value (about 50 mEq/l), as also shown in Figure 1. Because the sodium concentration in the chyme is normally about 142 mEq/l (that is, about equal to that in plasma), sodium moves down this steep electrochemical gradient from the chyme through the brush border of the epithelial cell into the epithelial cell cytoplasm. This provides still more sodium ions to be transported by the epithelial cells into the paracellular spaces (Guyton, 2006a).

Figure 1. Absorption of sodium through the intestinal epithelium. Note also osmotic absorption of water—that is, water "follows" sodium through the epithelial membrane



As indicated above, in rats (and humans), the intestinal absorption of ingested phosphorus is greatest in the jejunum and decreases along the length of the small intestine. A portion of phosphorus absorption takes place by a saturable, active transport which is facilitated by 1,25-hydroxyvitamin D. However, as phosphorus absorption occurs across a broad range of intakes, the bulk of absorption is mediated by passive, concentration-dependent processes. Changes in dietary phosphorus levels are known to affect the active transport of phosphate ions across the intestinal microvilli. Phosphorus transport is decreased when dietary phosphorus is increased and enhanced in chickens fed a low phosphate diet for five to seven days (FSA, 2002).

The mechanism of phosphate transport across the distal colon epithelium of normal adult rats has been examined (Hu, 1997) A 5-7 cm segment of distal colon, cut along the mesenteric border, was obtained from male adult Wistar rats fed normal, vitamin D-replete rodent diet (containing 1.2 % calcium and 0.8 % phosphorus) and water *ad libitum*. The segment was mounted across lucite chambers that exposed a 0.67 cm^2 of epithelium. The *in vitro* transmural phosphate and mannitol fluxes were studied, using modified Krebs-Ringer-HCO_3 buffer (containing 143 mmol/l sodium, 1.25 mmol/l calcium, 1.18 mmol/l phosphorus, 1.20 mmol/l magnesium, 2.0 mmol/l mannitol and 11.0 mmol/l *D*-glucose). Ion fluxes were calculated following liquid scintillation counting, using ^{32}P tracers as carrier-free orthophosphoric acid and ^{3}H-mannitol. Samples (1 ml) were taken at 15 minute intervals for

120 minutes from the initial ´cold` compartment and replaced by an aliquot of identical buffer. A steady-state flux rate was generally attained by 60 minutes. Equal volume of the solvent (normal saline) was added to the mucosal compartment of the control tissue and the serosal compartment of both experimental and control tissues. Transepithelial potential difference was abolished using automatic voltage-current clamp. Conductance was monitored at 15 minute intervals by recording the voltage required for the current-clamp to produce a 10 µA increase in current. Conductance was calculated as the difference between the unidirectional mucosal-to-serosal flux and the serosal-to-mucosal flux measured across two pieces of adjacent colon. The unidirectional net flux rates (mucosal-to-serosal flux minus the serosal-to-mucosal flux) from six studies (mean of flux rate measured at 60, 75, 90, 105 and 120 minutes) were 4.6 ± 1.8 nmol/cm²/h for phosphate and 4.6 ± 2.2 nmol/cm²/h for mannitol. To establish whether this similarity was due to phosphate transversing the colonic epithelium through the mannitol pathway, the permeability of the tight junction was increased by exposing the mucosal surface to taurodeoxycholic acid (final concentration 2 mM) at 60 minutes (below the *in vivo* concentration of bile salts in intestinal content and similar to the concentration previously used in studies on the effects of bile salts on electrolyte transport in rat colon). The increase in tight-junction permeability was confirmed by an immediate marked increase in tissue conductance. This increase in conductance was paralleled by an increase in serosal-to-mucosal flux for mannitol without significant changes in mannitol mucosal-to-serosal flux, resulting in a net secretory flux for mannitol. The pattern of changes in fluxes for phosphate following taurodeoxycholic acid treatment duplicated those observed for mannitol. A significant correlation also was observed between the permeability of phosphate and that of mannitol, both in the mucosal-to-serosal and the serosal-to-mucosal directions for control and taurodeoxycholic acid-treated epithelium. Based on these findings, it is probable that colonic phosphate transport was mediated through the same paracellular pathway as mannitol. Therefore an enema with high phosphate concentrations (1760 times blood concentration) could trigger rapid and massive phosphate absorption through this diffusive pathway.
Evidence from a number of human studies suggests that only limited systemic absorption of phosphate occurs when a mixture of monobasic and dibasic sodium phosphate is taken orally.

It has been reported that around 21 % of the sodium phosphate is absorbed by the colonic mucosa following the use of a dibasic and monobasic sodium phosphate disposable enema in preparing patients for sigmoidoscopy (Knoernschild, 1962).

Transport of phosphate from the gut lumen is modified by several factors including vitamin D, which stimulates absorption. The presence of large quantities of Ca^{2+} or Al^{3+} (as in sustained abuse of aluminium containing antacids) may lead to formation of large amounts of insoluble phosphate and diminish net phosphate absorption and can result in clinical phosphate depletion (Marcus, 2001).

When a five minute enforced retention was imposed on healthy cats after a commercial hypertonic sodium phosphate enema had been administered at the lower (60 ml, 18 ml/kg) and upper (120 ml, 32 ml/kg) doses recommended for this species (see Section II.2.2), all treated cats displayed dose-related hyperphosphataemia and hypocalcaemia,

hypernatraemia and hyperglycaemia (Atkins, 1985). Both treated groups also exhibited a calculated hyperosmolality and metabolic acidosis. It was evident that the enforced prolonged retention of sodium phosphate in this study resulted in absorption sufficient to markedly disturb serum electrolytes. As peritoneal absorption can result from perforations in the colon, the absorption of sodium phosphate following intraperitoneal administration was investigated in albino rats and has been reported (Lochbühler, 1995). A ready-made enema solution (Practo-Clyss, Schiwa) containing 160 mg/ml monobasic sodium phosphate and 60 mg/ml dibasic sodium phosphate was administered intraperitoneally at doses of 10, 12, 13, 14, 15 and 20 ml/kg. Controls received 20 ml/kg of sorbit solution at the same osmolality as the sodium phosphate solution (2500 mosm/l). The results indicated that the volume of the test substances, administered in a few seconds, overloaded the peritoneal drainage system, producing ascites. The absorption of sodium phosphate from the peritoneal cavity was evident from a dose-related hyperphosphataemia, clinical signs and deaths in all treated groups. Hypernatraemia, hyperkalaemia and hypocalcaemia also were reported in rats receiving 12 ml/kg sodium phosphate or above.

Plasma phosphate was determined in New Zealand White rabbits following intravenous infusion of a mixture of monobasic (0.55 % w/v) and dibasic (2.95 % w/v) sodium phosphate (Moore, 1988). Two groups, each containing four rabbits, were infused intravenously at a rate of 50 ml/hour for one hour to provide a total dose of phosphate approximating to the equivalent of 4 or 8 g/hour in humans. A further two groups (ten rabbits per group) served as sham and saline controls (50 ml/hour). A ´constant infusion` group (eight rabbits) was infused intravenously with a 1:2 dilution of stock phosphate solution at a rate of 15 ml/hour for three hours to investigate phosphate clearance. Blood samples were obtained before infusion and at 0.5 hour intervals from the start of infusion to four hours after completion of the infusion. Before each sampling, ECG traces were recorded from each rabbit. The results showed a rapid dose-related rise in plasma phosphate levels, accompanied by a parallel fall in calcium concentrations. Both phosphate and calcium plasma concentrations returned to normal by four hours post-infusion. The apparent volume of distribution for phosphate, infused for three hours, was 621.4 $\pm$ 42.7 ml. Corresponding estimates of Maximal velocity (V_{max}) was 0.10338 ± 0.043 mM phosphate/hour and the Michaelis constant ($K_{m)}$ was 2.1718 ± 0.0947 mM phosphate.

III.2.2 Distribution

Once absorbed, the sodium ion is rapidly distributed throughout the body. The concentration of sodium in blood and other extracellular fluids is about 145 mM (3,335 mg/l), whereas the concentration of sodium ion inside cells is about 12 mM (276 mg/l). This unequal distribution of sodium between extracellular and intracellular compartments is essential to the normal functioning of all cells and tissues of the body (US EPA, 2003).

Phosphate is an essential component of all body tissues and is a dynamic constituent of intermediary and energy metabolism. The normal body content of phosphorus in humans is approximately 700 g (23000 mmol), 80 % of which is found in bone. About 70 % of the phosphorus of blood is a constituent of phospholipids; the remainder is present as inorganic

phosphates, about 85 % free and 15 % protein-bound. Total phosphorus concentration in whole blood is 13 mmol/l, most of which is in the phospholipids of red cells and plasma lipoproteins. The intracellular-extracellular soluble phosphate ratio is estimated at 100:1 (FSA, 2002).

In extracellular fluid the bulk of phosphate exists as monobasic sodium phosphate (NaH_2PO_4) and dibasic sodium phosphate (Na_2HPO_4); in humans the ratio of dibasic to monobasic sodium phosphate is 4:1 at pH 7.4 (Marcus, 2001). The natural concentration of plasma inorganic phosphate varies with age, the values for newborn infants >children >adults; probably due to the lower glomerular filtration rate in infants (FSA, 2002). A reduction of plasma phosphate concentration permits the presence of more calcium in the blood without mineral precipitation.

III.2.3 Metabolism

The phosphorus and calcium balance within the body is normally maintained by parathyroid hormone, which decreases serum phosphorus concentration by an increase in urinary excretion. Parathyroid secretion can be stimulated by high serum phosphate concentrations in rats (FSA, 2002) and dogs (Estepa, 1999).

The phosphate anion, which is progressively concentrated in the renal tubule, represents the most abundant buffer system in the distal tubule. At this site, the secretion of H^+ by the tubular cell in exchange for Na^+ in the tubular urine converts dibasic to monobasic sodium phosphate, allowing large quantities of acid to be excreted without lowering the pH of the urine to a degree that would block H^+ transport by a high concentration gradient between the tubular cell and the luminal fluid (Marcus, 2001).

III.2.4 Excretion

Sodium is excreted mainly in the urine, although some sodium loss occurs with faecal matter and in perspiration. The kidney, nervous system, and endocrine system maintain very precise control of renal sodium excretion, with approximately 95 % to 98 % of the sodium being reabsorbed in the kidney. In the proximal tubule of the kidney, sodium resorption is coupled with organic solutes and anions and protons. Entry into the proximal tubule epithelium is mediated by symporter (e.g., Na^+-glucose, Na^+-$PO4^{-3}$, Na^+-lactate, and Na^+-amino acid symporters) and antiporter (Na^+-H^+ antiporter) proteins located on the apical membrane of the proximal tubule. When sodium enters the cytoplasm of the proximal tubule, it is actively transported into the blood by the Na^+/K^+-ATPase pump. Similar sodium resorption mechanisms occur in the loop of Henle and distal tubule (US EPA, 2003).

Phosphate in animals and humans is almost entirely excreted in the urine, the kidney being the major site for the regulation of the amount of phosphates retained in the body (FSA, 2002). There is little evidence of tubular phosphate secretion in the mammalian kidney. Phosphate excreted in the urine represents the difference between the amount filtered and that reabsorbed. Expansion of plasma volume increases urinary phosphate excretion.

Although the major hormonal regulator of tubular phosphate reabsorption is parathyroid hormone, other factors including vitamin D, growth hormone, vasopressin, calcitonin, thyroid hormone, oestrogen and glucocorticoids are known to play a role. Parathyroid hormone and 1,25-dihydroxyvitamin D_3 have been shown to decrease phosphate reabsorption whereas growth hormone and thyroxine are known to increase reabsorption (FSA, 2002). The renal clearance of phosphate in rabbits following intravenous infusion of a mixture of monobasic (0.55 % w/w) and dibasic (2.95 % w/v) sodium phosphate has been reported (Moore, 1988). The renal phosphate clearance, a function of both renal tubular reabsorption capacity (TmP) and glomeruler filtration rate (GFR), was shown to be greater at the high dose than at the low dose (approximating to the equivalent of 8 and 4 g/hour, respectively, in humans).

III.3 Toxicology

III.3.1 Single-dose toxicity

The effects of rectal administration of a single enema in adult cats have been reported (Atkins, 1985; Atkins, 1987). Groups of five healthy adult cats received a single administration of a commercial hypertonic sodium phosphate enema at the lower 60 ml (18 ml/kg, range 13 to 26 ml/kg) and upper 120 ml (32 ml/kg, range 28 to 43 ml/kg) doses recommended for cats weighing 11.35 kg or less (see Section II.2.2). All cats were subjected to a five minute enforced enema retention period (comparable with the retention time reported in humans). Blood samples were taken and rectal temperature and heart rate were recorded at intervals up to 24 hours after administration. All treated cats displayed dose-related hyperphosphataemia and hypocalcaemia, hypernatraemia and hyperglycaemia. Both treated groups also exhibited a calculated hyperosmolality and metabolic acidosis. These abnormalities were noted as early as 15 minutes following administration of the enema solution. Although no cats suffered profound hypocalcaemia or tetany, one animal receiving 120 ml (32 ml/kg) died four hours after enema administration. It was evident that the enforced, prolonged retention of sodium phosphate in this study resulted in phosphate absorption sufficient to markedly disturb serum electrolytes.

As the gastrointestinal physiologic characteristics of the pig are similar to those of the human, this species has been used to determine the effect of retained sodium phosphate enema solution (Martin, 1987). After fasting for 48 hours, seven female Yorkshire pigs (9.0 to 20.5 kg) were anaesthetised and maintained on 5% dextrose in 0.45% sodium chloride, infused at 5 ml/kg/h. A Foley catheter was inserted into the rectum transanally and the 30 ml balloon inflated, to prevent escape of the enema solution. The sodium phosphate solution supplied was in adult- (118 ml) and paediatric-sized (66 ml) enemas (Fleet Co.), containing 143 mg/ml monobasic sodium phosphate and 53 mg/ml dibasic sodium phosphate. Doses in the range of 5 to 50 ml/kg were instilled through the Foley catheter, which was then clamped to provide 40 minutes enforced retention. The lowest dose (5 ml/kg) approximated to the dose for an average 2-year-old child (weighing 10 to 13 kg) receiving one paediatric-sized enema and the highest dose (50 ml) was equivalent to the fatal dose in the 11-month-old male infant (8.9 kg) described in a case report by the authors. Arterial blood samples, for electrolyte and pH

determinations, were collected at 20 minute intervals over a four-hour period *via* an indwelling catheter in the femoral artery. Pigs that survived one experiment were rested for at least seven days and then used for a repeated run.

One death occurred at dose of 20 ml/kg and all pigs died at doses of 30 ml/kg or more. It is likely that the LD_{50} for sodium phosphate enema in pigs, following rectal instillation and forced retention, would be close to 30ml/kg. Dose-related hyperphosphataemia and hypocalcaemia were evident at all doses. The changes were more marked at higher doses and included hypernatraemia and decreases in plasma pH. Other measurements, including serum chloride, potassium, urea nitrogen, glucose and haematocrit were undisturbed by treatment.

Similar phosphate enema toxicity has been described in a ruminant species (Hickman, 2004). A 13.6 kg, seven-month-old, castrated male, pygmy goat was treated with five adult Fleet phosphate enemas (five times the recommended adult-sized enemas for animals weighing 11.3 kg, or more). Each enema provided a delivered dose of 118 ml sodium phosphate solution, containing 19 g (161 mg/ml) of monobasic sodium phosphate and 7 g (59 mg/ml) of dibasic sodium phosphate. The enemas were instilled at a rate of one/hour over a five-hour period. The goat received a total dose of 590 ml (43 ml/kg), containing 95 g (a dose of 6.99 g/kg) monobasic sodium phosphate and 35 g (a dose of 2.57 g/kg) dibasic sodium phosphate. Clinical findings included mild depression, tachycardia (heart rate 144 beats/min, normal range 70-120 beats/min), tachypnoea (respiration rate 100 breaths/min, normal range 10-30 breaths/min), rumen stasis, muscle tremor, azotaemia (blood urea nitrogen 16 mmol/l, normal range 2.0-4.6 mmol/l), elevated blood creatinine (380 mmol/l, normal range 79.5-159 mmol/l), hyperphosphataemia (7.6 mmol/l, normal range 1.4-3.0 mmol/l), hypocalcaemia (0.95 mmol/l, normal range 2.1-2.6 mmol/l), hypochloraemia (94 mmol/l, normal range 105-120 mmol/l), hypokalaemia (3.3 mmol/l, normal range 4.6-6.7 mmol/l) and metabolic acidosis (TCO_2 21.9 mmol/l, normal range 26-30 mmol/l). Recovery was achieved after three days fluid diuresis and parenteral antimicrobial therapy.

III.3.2 Repeat-dose toxicity

The effects of excess oral sodium phosphate on silica urolith formation were investigated in dietary studies in male Sprague-Dawley rats fed dextrose-based diets containing 0 or 2 % tetraethylorthosilicate for eight weeks (Schreier, 1986). In the first study six groups of 20 rats were fed diets containing either 0 or 2 % tetraethylorthosilicate. In rats receiving the tetraethylorthosilicate, urinary silica concentrations were generally in the range of 50-60 mg/dl and the incidence of silica urolith in the urinary bladder and kidneys was 35 %.

In diets containing tetraethylorthosilicate, the addition of 1 or 2 % calcium carbonate to the diet increased the urolith incidence to 45 and 60 %, respectively. The increase in urolith formation in rats fed calcium carbonate was associated with increased urine alkalinity and a reduction in urine phosphorus concentration. In a second study, six groups of 20 rats were fed diets containing either 0 or 2 % tetraethylorthosilicate. The effects of adding monobasic or dibasic sodium phosphate or a combination of dibasic sodium phosphate and sodium bicarbonate on urolith formation was assessed.

The addition of monobasic sodium phosphate to the diet (providing 0.2 % additional phosphorus) produced a mean urinary pH of 6.42 and no urolith formation was evident. The use of dibasic instead of monobasic sodium phosphate in the diet adjusted the urinary pH to 6.78 and small uroliths (incidence 15 %) were only observed in the tetraethylorthosilicate treated rats. Dibasic sodium phosphate plus 0.5% sodium bicarbonate in the diet shifted the urinary pH to 7.14 but uroliths were only evident in rats receiving tetraethylorthosilicate (incidence 20 %), although the size of uroliths remained small. In a third study casein (the protein source in studies one and two) was replaced with autoclaved egg albumin, which lowered the dietary phosphorus and increased urine alkalinity. Five groups of 24 rats received control diet or diet supplemented with an equal-molar mix of monobasic and dibasic sodium phosphate, ammonium chloride, a combination of ammonium chloride plus the monobasic and dibasic sodium phosphate mix or diammonium phosphate.

The increased alkalinity of the urine in the control group produced by the dietary egg albumin was associated with a urolith incidence of 46 %. By comparison, the urinary alkalinity of the group receiving the equal-molar mixture of monobasic and dibasic sodium phosphate, providing 0.2 % additional phosphorus, was reduced and the incidence of uroliths was lowered to 4 %. The groups receiving 0.75% of dietary ammonium chloride (with or without the 0.2 % additional phosphorus provided by the mixture of monobasic and dibasic sodium phosphate), ensured urine acidification and no uroliths were formed. Dietary diammonium phosphate, although less effective at acidifying the urine, did reduce the incidence of uroliths to 12 %.

The effect of excessive dietary sodium phosphate on kidney and parathyroid tissue in rats has been reported by (FSA, 2002). Mature male rats (strain and numbers not specified) were fed diet containing a high level of sodium phosphate (8 % in diet, approximately 4 g/kg/day), providing about 1 g/kg/day elemental phosphorus or 38 mM/kg/day, for up to seven months. Microscopic examination of the tissues at the time of death showed that calcium deposits were present in the tubules of the kidneys and other organs. Hypertrophy and hyperplasia were also evident in parathyroid cells. In addition, the long bones of the treated rats appeared thickened and more fragile than those of control rats. An investigation of phosphate-induced renal injury in uninephrectomised, partially nephrectomised and intact rats has been reported by (FSA, 2002). Groups of six rats received diets containing phosphate (form not specified) at concentrations of 0.5, 1 and 2 % (approximately 250, 500 and 1000 mg/kg/day) for 18 weeks. None of the intact animals receiving 250 mg/kg/day (a normal phosphorus intake) displayed any abnormalities. Four of the six intact rats receiving 500 mg/kg/day had normal kidney calcium concentrations; one animal showed histological changes in the kidney. All but one of the partial and uninephrectomised rats receiving 500 mg/kg/day phosphorus displayed increased kidney calcium concentrations and five of the six animals in the group displayed histological changes in the kidney.

A literature search provided details on the nephrotoxicity associated with intravenous administration of sodium phosphate solution to rats (Tsuchiya, 2004). Five groups of six male Sprague-Dawley rats received daily bolus intravenous administration of dibasic sodium phosphate in physiological saline at concentrations of 0, 1, 25, 250 and 360 mM (0, 1, 28, 284

and 408 mg/kg/day), at pH 5.5-6.5, for 14 days. Clinical signs and body weights were recorded daily, urine samples were collected over a four hour period after dosing on days 7 and 13 of treatment and blood samples were obtained before necropsy for blood chemistry evaluation. On completion of treatment tissues were examined macroscopically and retained for histological processing and microscopic examination. Kidney tissue was processed from two animals per group for electron microscopy. No abnormal clinical signs or body weight changes were evident and blood chemistry remained undisturbed, although mild to moderate proteinuria was evident from the urinalysis data at doses of 250 mM (284 mg/kg/day) or more. At necropsy, the kidney weights displayed no statistically significant differences between treated and control groups. The kidneys from rats receiving 360 mM were pale but no remarkable macroscopic lesions were seen. Histopathology examination revealed minimal focal deposition of fine basophilic granules in the glomerular and parietal epithelium in the 250 mM group. Panglomerular deposition of basophilic granules was observed in the 360 mM group with mild hypertrophy and vacuolisation of the parietal epithelium. Observations also included some glomeruli with rounded protuberances on the glomerular basement membrane. Calcium deposits, confirmed by von Kossa's staining, were reported in the basophilic granules and the glomerular basement membranes with rounded protuberances. Minimal calcification was seen in the basement membrane of some proximal tubules in the 250 and 360 mM groups. Electron microscopy indicated the presence of low density lamellar structures within the Bowman's space, glomerular epithelium, glomerular basement membrane, subepithelial spaces, mesangial matrix and parietal epithelium in the 250 and 360mM groups; these changes were more severe in rats receiving 360 mM. X-ray microanalysis of particles mixed with clusters of lamellar structures revealed peaks of calcium and phosphorus in the particles. Vacuoles of various sizes were observed within the glomerular epithelium, glomerular membrane, mesangial matrix and parietal epithelium. Fusion of podocytes, increase in the number of microvilli and large amounts of debris filling the Bowman's space were reported, depending on the severity of the glomerular lesions. It is likely that the proteinuria seen at 250 and 360 mM resulted from transient overloading of the glomerular epithelium during filtration through glomerular capillaries which produced insoluble calcium salts and glomerular lesions following these high doses of phosphate.

III.3.3 Genotoxicity

The effect of sodium phosphate buffers on the mutagenicity of two pure compounds and two complex mixtures and the cytotoxicity of these buffers in microsuspension reverse mutation assays has been investigated (DeMarini, 1989). The assay was performed using *Salmonella typhimurium* strain TA98 in 0.15 M or 0.015 M sodium phosphate buffer, pH 7.4 or Vogel-Bonner minimal E buffer (VBM). Environmental tobacco smoke (which requires the S9 mix metabolising system) and diesel exhaust (mutagenic in the absence of S9 mix) were used as the two complex mixtures. The two pure compounds selected were 2-aminoanthracene (requires S9 mix) and 1-nitropyrene (does not require S9 mix), these being representative of the classes of chemicals that accounted for a majority of the mutagenic activity of environmental tobacco smoke and diesel exhaust, respectively.

The buffers were equally suitable in the presence of S9 mix but 1-nitropyrene was considerably more mutagenic in 0.015 M sodium phosphate compared with 0.15 M sodium phosphate or VBM. The mutagenic potency was more complex for diesel exhaust, depending on cell concentration as well as buffer type. The cytotoxicity of the buffers was assessed from the relative survival of T98 after 90 minutes incubation compared to before incubation with the buffers with or without S9 mix.

The 0.15 M sodium phosphate buffer and VBM exerted considerable cytotoxicity, in the absence of S9 mix, resulting in an under-estimate of the mutagenic potency of the directly acting mutagens 1-nitropyrene and diesel exhaust. At a lower concentration (0.015 M) phosphate buffer had only a weak cytotoxic effect and did not interfere with the evaluation of known mutagens. The addition of S9 mix reduced the cytotoxicities of the buffers. The cytotoxicities of the buffers in the absence of S9 mix were investigated further by determining the number of surviving cells after incubation at various concentrations of sodium phosphate and calculating the relative cell survival by comparison with those obtained with 0.015 M sodium phosphate (100 %).

Increased sodium phosphate concentrations resulted in decreased cell survival.
Neither 0.15 M nor 0.015 M sodium phosphate buffer provided any evidence for an intrinsic mutagenic action in these investigations.

III.3.4 Carcinogenicity

A study on the long-term effects of dietary phosphoric acid in three generations of rats was investigated by (FSA, 2002). Although a limited older study by today's standards, it does provide some relevant information on the long-term effects of ingested phosphorus. Rats received diets containing 0.4 and 0.75 % phosphoric acid (approximately 200 and 375 mg/kg/day, respectively) for 90 weeks. Growth and reproduction were not adversely affected by treatment and there were no significant differences in haematology parameters between treated and control groups. No pathological findings were attributable to treatment. Calcium metabolism remained unchanged and there was no indication of acidosis in the treated groups, although dental attrition was more marked in treated rats than in controls.

III.3.5 Reproductive and developmental toxicity

Only one embryo-foetal development study was identified. The potential of sodium phosphate to induce embryotoxic and teratogenic effects has been assessed in chicken embryos (Korhonen, 1983). Three day (72 to 76 hours) White Leghorn chicken egg embryos, 20 to 30/group selected by candling, received injections (total volume 5 µl) on the inner shell membrane, on to the embryo heart and into the air chamber of the egg at doses of 0, 4, 8 and 16 µmol/egg monobasic sodium phosphate. Ten control eggs were injected with 5 µl acetone and incubated with each batch of eggs. The eggs were incubated and turned two to four times each day. Two days after injection eggs containing dead embryos, determined by candling, were counted and discarded. Eggs were subsequently candled every second or third day. Those containing dead embryos were opened and the embryos were checked for their

developmental stage and any external malformations. Incubation was terminated on day 14 (11 days after injection). The eggs were opened and the embryos examined for survival and external malformations. The solvent background of controls was insignificant and was not subtracted from experimental values.

Monobasic sodium phosphate was relatively non-toxic, with similar values for total mortality LD_{50} (12 µmol/egg), early deaths LD_{50} (13 µmol/egg), both approximated on probit paper, and total embryotoxicity ED_{50} (11 µmol/egg). Even at embryotoxic doses monobasic sodium phosphate did not cause any increased incidences of malformed embryos. Of the 4 malformed survivors (3 at 8 µmol/egg and 1 at 4 µmol/egg), the frequency of the different malformation types were 3 (75 %) open coelom, 2 (50 %) back and neck and 1 (25 %) oedema. These data suggest that monobasic sodium phosphate lacks teratogenic activity.

III.3.6 Local tolerance

As expected for materials that are ubiquitously distributed in living cells, the active components of Cleen enema (dibasic and monobasic sodium phosphate) are well tolerated and show no significant indication of activity in the rabbit skin and eye irritation tests (Weiner, 2001).

III.3.7 Other toxicity studies

Sodium phosphate enemas have been used to ensure adequate colon cleansing before examining the colon of patients with suspected inflammatory bowel disease. An investigation has been performed to establish whether the use of a sodium phosphate bowel cleansing product could affect the morphology of the rectal mucosa in rats with chronic colitis (Erdogan, 2003). Groups of ten healthy female Sprague-Dawley rats received intrarectal administrations of either 1 ml of 0.9% saline (healthy control) or 0.35 ml of sodium phosphate solution (Fleet Co. Inc.) under light anaesthesia following overnight fasting. A second treated group, without anaesthesia, received two 10 ml oral (gavage) administrations of polyethylene glycol-electrolyte (Sigma Chemical Co.). A further three groups of 8 to 9 rats received saline (colitis control), sodium phosphate or polyethylene glycol-electrolyte, as described above, 14 days after the induction of colitis by rectal instillation of 0.25 ml of 2,4,6-trinitrobenzene sulfonic acid (TNBS) dissolved in 50 % ethanol (a dose of 30 mg/kg TNBS), using the procedure described above (the optimum period for producing chronic inflammation and ulceration was one to two weeks after administration of TNBS). Eight hours after the administration of saline, sodium phosphate or polyethylene glycol-electrolyte, each animal was sacrificed and the distal colon was removed for macroscopic evaluation for damage. The changes were assessed, using a scoring system of 0 (no damage), 1 (localised hyperaemia but no ulcers), 2 (linear ulcers with no significant inflammation, *i.e*. regions of hyperaemia and bowel wall thickening), 3 (linear ulcer with inflammation at one site), 4 (two or more sites of ulceration and/or inflammation) and 5 (two or more major sites of inflammation and ulceration or one major site of inflammation and ulceration extending more than 1cm along the length of the colon). Three tissue samples (2 x 2 mm) were taken from each colon, including any ulceration or inflammation sites, for processing (including periodic acid-Schiff stain for identification of

mucus concentration) and microscopic evaluation. Scoring criteria from 0 (normal morphology) to 3 (severe changes) were used to assess the microscopic changes with respect to ulceration, mucus cell depletion crypt abscesses, inflammatory cysts, mucosal atrophy, oedema (submucosa), inflammatory cell infiltration and vascular dilation.

Although aphthoid lesions were seen macroscopically in healthy treated rats, three animals (incidence, 30%) given sodium phosphate and 2 animals (incidence, 20%) given polyethylene glycol-electrolyte, no significant difference was found among the three healthy rat groups. Similarly, no significant difference was found among the three TNBS-induced colitis groups. As expected, there was a significant difference between the TNBS-induced colitis groups and their equivalent healthy rat groups ($p < 0.001$). Microscopic evaluation of aphthoid lesions observed in a few healthy rats receiving sodium phosphate or polyethylene glycol-electrolyte revealed oedema within the lamina propia and lymphoid hyperplasia in the mucosa and submucosa without erosion, ulceration and inflammatory cell infiltration. Focal active colitis was found in one healthy rat (incidence 10%) given sodium phosphate. In healthy rats, there was a significant difference in the scores for submucosal oedema and vascular dilatation in the sodium phosphate group compared with the saline controls but no statistical difference in the scores between polyethylene glycol-electrolyte and saline control groups. No significant difference was found among the three TNBS-induced colitis groups. As expected, there was a variety of significant differences in the severity of the lesions between the TNBS-induced colitis groups and their equivalent healthy rat groups. The authors concluded that sodium phosphate caused no evident morphological changes on the rectal mucosa either macroscopically or microscopically in healthy and colitis groups of rats.

III.4 Ecotoxicity/environmental risk assessment (ERA)

Since Cleenema is intended for generic substitution, this will not lead to an increased exposure to the environment. An environmental risk assessment is therefore not deemed necessary.

III.5 Discussion on the non-clinical aspects

The submission is intended for well-established use. As such, the MAH has not provided additional non-clinical studies and further studies are not required. An overview based on literature review is, thus, appropriate. The effects of sodium dihydrogen phosphate dihydrate and sodium phosphate dodecahydrate are well known, and the literature on pharmacology, pharmacokinetics and toxicology has been adequately reviewed in the MAH's non-clinical overview.

IV. CLINICAL ASPECTS

IV.1 Introduction

Sodium dihydrogen phosphate dihydrate and sodium phosphate dodecahydrate 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.

As Cleenema is intended as a well-established use medicinal product, the MAH has submitted a literature review encompassing the pharmacokinetics, where pharmacokinetic data of Cleenema was bridged to studies using morphine hydrochloride/sulphate formulations. Pharmacodynamics, clinical efficacy and safety have also been linked to clinical studies and are considered to be adequate.

IV.2 Pharmacokinetics

Although only a local effect is intended, part of the phosphate can be absorbed. This can lead to mild hyperphosphatemia, which is acceptable. In a study (Peery *et al.*, 2005) in which two sodium phosphate enemas (Enema Casen 250 ml, Laboratorios Casen-Fleet, S.A., Spain, 40 g monobasic NaP monohydrate, 20 g dibasic NaP dodecahydrate; Fleet Enema 133 ml, 19 g monobasic NaP monohydrate, 7 g dibasic NaP heptahydrate) were used, 30% of subjects had mild hyperphosphatemia (4.5 - 7.0 mg/dL), which peaked at 10 minutes after enema evacuation (the increase was transient). The amount of increase was related to the retention time of the enema fluid, but not the enema size.

In addition, sodium can be absorbed from phosphate enemas. In Peery *et al.* (2005), sodium remained within normal clinical limits for all subjects, except for one subject, with type II diabetes, 2 hours post evacuation.

As stated above, sodium and phosphate from the proposed product can be absorbed. As generally known, these ions will distribute over the body fluids.

Fine and Patterson (1997) concluded that although safe in the vast majority of patients, sodium phosphate administration for cathartic purposes should be considered potentially dangerous in the presence of renal impairment, especially with multiple doses. In the proposed SmPC, the applicant has written precautions for patients with renal impairment. It states that the product must not be administered to patients with clinically significant impairment of renal function. Furthermore, it states that the product should be used with caution in patients with impaired renal function, when the clinical benefit is expected to outweigh the risk of hyperphosphatemia. From a pharmacokinetic point of view this is acceptable.

<u>Bridging of reported pharmacokinetics in literature to Cleenema enema</u>
The quantity of anhydrous phosphates per mL in the proposed product (133 mL), and in the existing products Fleet Enema (133 mL) and Enema Casen (250 mL) is practically the same. Jacobson et al. (2010) also found that “Increases during the first 10 minutes were similar for both enema cohorts [Fleet Enema 133 mL and Enema Casen 250 mL], so the

hyperphosphatemia was not dose-related. This suggests a saturable absorption process.". Therefore, the pharmacokinetic results of the study by Jacobson et al. (2010) can be bridged to the proposed product.

IV.3 Pharmacodynamics

The cathartic action of enemas with disodium hydrogen phosphate and sodium dihydrogen phosphate as active substances is believed to result from osmotically mediated water retention which then stimulates peristalsis and bowel movement with only the rectum, sigmoid and part, or all, of the descending colon being evacuated (Goodman and Gilman, 2018; Marks, 1951; Bevilacqua, 1959). Phosphate enemas act within two-five minutes (Martindale, 2009).

It is commonly believed that the action of saline laxatives results from a hyperosmotic effect of poorly absorbed magnesium or phosphate ions within the small intestine, and increased water retention, which indirectly stimulates stretch receptors and increases peristalsis. These mechanisms of action are however unproven, and conversely, isosmolarity at the level of the ligament of Treitz has been shown following the ingestion of a hyperosmolar meal. Increased water content in the colonic lumen causes an increased stool volume, distension of the gut wall, and increased peristalsis due to activation of local neural reflexes.

Mono and dibasic sodium phosphates are characterized by a slow and incomplete absorption from the gastrointestinal tract, and their presence in the large intestine causes water retention in the intestinal lumen as a result of osmotic forces.

The MAH submitted multiple publications to substantiate the pharmacodynamic effects of sodium phosphate enemas. Respective publications address a wide range of topics. Some of these publications concern the mechanism of action of sodium phosphate laxatives (Steinberg & Almy 1965, Kemp 1971). Other publications support the bowel cleansing pharmacodynamic properties of sodium phosphate laxatives with respect to bowel cleansing in the distal parts of the gastrointestinal tract (Riley & Natvig 1966, Tillary & Bates 1966, Knoernschild & Cameron 1962).

Altogether, submitted publications support the pharmacodynamic action of sodium phosphate enemas.

IV.4 Clinical efficacy

IV.4.1 Occasional constipation

Information from a textbook (Goodman & Gilman 2018), a guideline (Prodigy 2005), reviews (UpToDate sodium phosphate 2019, Sobrado et al. 2018, Ferry 2014, Pasricha 2006, Davies 2004, Brandt 1987, Marks 1951), retrospective studies (Anderson et al. 2019, Librizzi et al. 2017, Hansen et al. 2011), and an observational study (Eidlitz-Markus 2004) were submitted

to substantiate the efficacy of sodium phosphate enemas to induce relief of occasional constipation. The scientific evidence for this efficacy is summarized below by literature source.

Textbook
Goodman & Gilman (2018) indicated that the mechanism of action of saline laxatives such as sodium phosphate enemas is believed to result from osmotically mediated water retention, which then stimulates peristalsis. The authors remarked that other mechanisms may contribute to their effects, including the production of inflammatory mediators.

Guideline
Prodigy (2005) states that osmotic laxatives such as phosphate enemas act by retaining fluid in the bowel by osmosis or changing water distribution in faeces. The author indicated that rectal phosphate preparations are useful when quick relief is desirable. The author also stated that phosphate enemas are most often used for bowel clearance prior to surgery of bowel investigations. A good fluid intake should be encouraged.

Reviews
Pasricha (2006), Davies (2004), and Marks (1951) made clear that phosphate enemas are used for laxative purposes. In the publication by Pasricha (2006), it is stated that such preparations are tolerated reasonably well by most patients. However, they must be used with caution or avoided in patients with renal insufficiency, cardiac disease, or pre-existing electrolyte abnormalities, and in patients on diuretic therapy. Pasricha (2006) also warns for the occurrence of electrolyte shifts that pose a risk for the development of symptomatic dehydration, renal failure, metabolic acidosis, tetany from hypocalcaemia, and even death in medically vulnerable populations.

Despite the above, Brandt (1987) indicated that an acute episode of faecal impaction or constipation can usually be treated safely with enema therapy. In line with this, Sobrado et al. (2018) state that enemas or suppositories may be used in chronically constipated patients (e.g., those with psychogenic megacolon) or faecal impaction, in which the initial measures (fiber, fluids and laxatives) were ineffective. Transanal irrigation by sodium phosphate or vegetable oils should be limited to brief periods.

Ferry (2014) stated that paediatric patients with faecal impaction may be treated with a sodium phosphate enema (using the appropriate sized enema for the child's age), followed by one or two doses of a laxative. This author also indicated that toddlers and children with recurrent constipation should be treated with a course of laxatives, increasing fibre intake in the diet or with supplements (using a higher target of 14 g/1000 kcals in the diet, which translates to approximately 20 g/day in early childhood), and/or faecal disimpaction if necessary (using a sodium phosphate enema).

According to UpToDate (2019) recommended (Fleet) laxative dosing increases with age: one-half contents of one 2.25 oz paediatric enema in children aged two-four years, one 2.25 oz paediatric enema in children aged 5-11 years, and one 4.5 oz enema as a single dose in patients aged 12 years and above.

Retrospective studies
In retrospective study by Anderson et al. (2019) 768 patients who had received at least one enema in the emergency department during the prior five-year study period were included in the study. There was no statistically significant association between reported stool output (small, medium, or large) and enema solution for the three most frequent enema types, sodium phosphate, pink lady, and soap suds (p=0.88). Most frequently reported side effects of enema were abdominal pain and vomiting (both 0.5% of patients).

Librizzi et al. (2017) reported on 14,243 hospitalizations of 12,804 unique patients. The authors noted that there was a wide variation among hospitals in the administration of sodium phosphate enemas (0 – 64%). According to NASPGHAN clinical guidelines sodium phosphate enemas and polyethylene glycol are equally effective in treating rectal faecal impaction in the outpatient setting.

In a retrospective study by Hansen et al. (2011) milk and molasses enemas and sodium phosphate enemas were equally efficacious and safe for the treatment of constipation in the paediatric emergency department.

Observational study
In observational study by Eidlitz-Markus (2004) occult constipation was in 42.6% of examined paediatric patients the cause of recurrent abdominal pain. Upon treatment with paraffin oil and phosphate enemas, the abdominal pain subsided considerably or disappeared within 2 weeks to 3 months of treatment in 82.84% of patients. At a telephone interview at 1±1.5 years after discharge, abdominal pain and constipation had subsided or disappeared in 96.5% of paediatric patients according to their parents.

Above documentation supports the short-term efficacy of sodium phosphate laxatives such as enemas for relief of occasional constipation in paediatric and adult patients. Across different studies, the clinical effects of sodium phosphate were comparable with those of pink lady, and soap suds (Anderson et al. 2019), polyethylene glycol (Librizzi et al. 2017), and milk and molasses enemas (Hansen et al. 2011). These findings are supported by some external literature (Bowers 2006).

<u>Dosing recommendation</u>
One bottle of 133 ml no more than once daily is recommended to induce relief of occasional constipation in adults.

The MAH has not substantiated this particular dosing recommendation for this indication. However, at least one bowel movement occurred in all adult study patients after administration of either a Fleet sodium phosphate enema (133 ml; n= 20) or a Casen sodium phosphate enema (250 ml; n= 25) in submitted study by Jacobson et al. (2010). The chemical composition of proposed sodium phosphate enema is the same as the 133 ml Fleet sodium phosphate enema described in literature. Hence, appropriate bowel cleansing will also be obtained upon administration of one 133 ml unit of proposed sodium phosphate enema.

For the relief of occasional constipation in adults a similar posology is recommended in currently proposed SmPC and the SmPCs of several authorized sodium phosphate enemas within the European Union (e.g. Colex enema solution for rectal use (NL/H/0619/001), Cleenema read-to-use 21.4g/9.4 g enema (marketing authorization number Ireland: PA 2028/1/1)). Because of this, proposed posology for the relief of occasional constipation in adults is considered acceptable.

IV.4.2 Preparation of medical and diagnostic procedures, if needed

Submitted scientific evidence on the clinical efficacy of sodium phosphate on an if needed base for preparation of medical and diagnostic procedures will in this assessment report be assessed with respect to the following particular entities:

- Bowel cleansing before and after lower bowel surgery,
- Bowel cleansing before proctoscopy and sigmoidoscopy,
- Bowel cleansing before radiological examinations of the lower bowel.

<u>Bowel cleansing before and after lower bowel surgery</u>

A review (Huang et al. 2006), three open label studies (Aslan et al. 2013, Jacobson & Thompson 2004, Yang et al. 2011), and a survey (Shpitz et al. 2005) were submitted in order to substantiate the clinical efficacy of sodium phosphate enemas for bowel cleansing before and after lower bowel surgery.

Review

In a review by Huang et al. (2006) patients who had had a prostate biopsy were reviewed retrospectively. Group 1 consisted of patients who self-administered a phosphate enema at home (n= 65). Group 2 had a phosphate enema combined with povidone-iodine administered by a doctor at the hospital (n= 157). In group 1, six patients (9.23%) had a symptomatic infection with leukocytosis or chills. None were found in group 2. The authors concluded that phosphate enema with povidone-iodine administered at the hospital is an effective way to reduce the infection rate for agricultural people who have poor compliance or inaccuracy.

Open label studies

Aslan et al. (Aslan et al. 2013) investigated the outcomes and complication rates of urinary diversion using mechanical bowel preparation (BP) with three day conventional and limited BP method through a standard perioperative care plan. This study was designed as a prospective randomized multicentre trial. All patients were randomized to two groups. Patients in standard three-day BP protocol received diet restriction, oral antibiotics to bowel flora, oral laxatives, and saline enemas over a three-day period, whereas limited the BP arm received liberal use of liquid diet, sodium phosphate laxative, and self-administered enema the day before surgery. All patients received same perioperative treatment protocol. Fifty-six patients in three-day BP and 56 in limited BP arm were evaluable for the study end points. Postoperatively, one patient in limited BP and two patients in three-day BP arm died. There was no statistical difference in any of the variables assessed throughout the study, however,

a favourable return of bowel function and time to discharge as well as lower complication rate were observed in limited BP group.

Jacobson & Thompson (2004) evaluated the changes of serum electrolytes over a two-hour period following the administration of a Fleet sodium phosphate enema (133 ml), or a Sodium phosphate enema Casen (250 ml) in healthy adults. In order to properly assess efficacy, subjects were asked to retain the enema fluid as long as possible, up to ten minutes. The time to bowel movements following the administration of the enema was recorded for each subject. An enema was considered effective if a bowel movement occurred after its administration. All subjects had at least one bowel movement, occurring between 30 seconds and ten minutes following the administration of the enema, thus, both enemas were considered effective. Mean retention times did not differ between the two enemas (p=0.9144), The mean retention time was 5.7 minutes in the 25 patients receiving Enema Casen, and of 6.0 minutes in the 20 patients receiving Fleet Enema.

Yang et al. (Yang et al. 2011) compared the effect of mechanical bowel preparation using oral sodium phosphate (NaP) solution vs. single NaP enema on the quality of the surgical field in patients undergoing advanced gynecologic laparoscopic procedures. One hundred fifty-six women were enrolled, and 145 were randomized to receive either oral NaP solution (n = 72) or NaP enema (n = 73). Sixty-eight women in the oral solution group and 65 in the enema group completed the study. Assessment of the quality of the surgical field and bowel characteristics was performed using a surgeon questionnaire using Likert and visual analogue scales. No significant differences were observed between the two groups in evaluation of the surgical field, bowel handling, degree of bowel preparation, or surgical difficulty. Surgical field quality was graded as excellent or good in 85% of women in the oral solution group and 91% of women in the enema group (p = 0.43). When surgeons were asked to guess the type of preparation used, they were correct only 52% of the time (κ = 0.04). Assessment of patient quality of life in the preoperative period was performed using a self-administered questionnaire using a visual analogue scale. Severity of abdominal bloating and swelling, weakness, thirst, dizziness, nausea, fecal incontinence, and overall discomfort were significantly greater in the oral solution group. Women in the oral solution group also rated the preparation as significantly more difficult to administer and were significantly less willing to try the same preparation in the future. The authors concluded that quality of the surgical field in patients undergoing advanced gynecologic laparoscopic procedures is similar after mechanical bowel preparation using either oral NaP solution or NaP enema. Adverse effects are more severe with oral NaP solution compared with NaP enema administration.

Survey

Shpitz et al. (Shpitz et al. 2005) performed a national survey where they found that in a first survey, four departments from this Survey (17%) used Fleet enema or laxative suppositories in addition to oral bowel preparation. Two departments used neomycin enema. In a second (and more recent) survey, ten departments (34%) used monobasic/dibasic sodium phosphate enema (Fleet®, C.B. Fleet, Inc., Lynchburg, VA) and none used neomycin enemas.

Limited scientific evidence with respect to the efficacy of sodium phosphate enemas for bowel cleansing prior to or after lower bowel surgery. No publications were submitted to substantiate the efficacy of sodium phosphate enemas for bowel cleansing after a surgical procedure.

Bowel cleansing before proctoscopy and sigmoidoscopy

A consensus document (Wexner 2006), reviews (A-Rahim & Falchuk 2014, Skalnik 1978), randomized controlled studies (Gidwani et al. 2007, Atkin et al. 2000, Fincher et al. 1999, Lund et al. 1998, Osgard et al. 1998, Drew et al. 1997, Underwood et al. 2010), an open-label study (Jacobson & Thompson 2004), an observational study (Gross et al. 1955), and an opinion statement (Nesselrod 1958) were submitted to substantiate bowel cleansing before proctoscopy and sigmoidoscopy.

Consensus document

Wexner (2006) indicated in the context of a consensus document on bowel preparation before colonoscopy prepared by a task force from the American Society of Colon and Rectal Surgeons (ASCRS), the American Society for Gastrointestinal Endoscopy, and the Society of American Gastrointestinal and Endoscopic Surgeons indicated that sodium phosphate enemas were found to be more or equally effective and better tolerated than with four litre PEG. A divided-dose sodium phosphate regimen in which the first dose is given the evening before the procedure and the second is given ten to 12 hours later on the morning of the procedure has proven to be more effective than a regimen using two doses of sodium phosphate given the day before the procedure or a regimen using full-volume (four-litre) PEG.

Review

According to the review by A-Rahim & Falchuk (2014) the preparation for flexible sigmoidoscopy typically involves two sodium phosphate enemas given the morning of the examination. Skalnik (1978) stated that enemas with plastic disposable tips may be used for bowel cleansing, since these tips are atraumatic to the gut.

Randomized controlled studies

In the study by Gidwani et al. (2007) 261 patients scheduled for out-patient flexible sigmoidoscopy were prospectively randomized to three groups: group 1 (n= 105): one Fleet enema two hours pre-procedure; group 2 (n= 81): two Fleet enemas, one on the evening prior to sigmoidoscopy and one two hours pre-procedure; group 3 (n= 75): lactulose 30 ml orally 48 and 24 hours prior to sigmoidoscopy, plus a single Fleet enema two hours pre-procedure. There was no significant difference between the groups in terms of depth of insertion (p=0.42-chi-squared test) or abnormalities noted (p=0.34-chi-squared test). Nor was there any difference in the quality of preparation of patients in group 1 versus group 2 (p=0.39-Fishers exact test) or group 1 versus group 3 (p=0.13-Fishers exact).

Atkin et al. (2000) evaluated the acceptability and efficacy of two methods of self-administered bowel preparation for flexible sigmoidoscopy screening: a single phosphate enema and a single sachet of Picolax. 1442 Men and women aged 55-64 years who had agreed to be screened by flexible sigmoidoscopy. Compliance with the enema was higher than with

the Picolax (608 (84%) vs 566 (79%); difference 6%, 95% confidence interval 2% to 10%). The quality of preparation was better with the enema; the proportion of procedures complete to the descending colon was greater and the mean duration of the procedure was shorter. There was no significant difference in polyp detection rates.

Fincher et al. (1999) reported that the preparation quality for sigmoidoscopy was excellent or good for 80.6% in the bisacodyl group, 88.7% in the one-enema group, and 85.1 % in the two-enema group (p=0.30). Hence, there was no statistical difference between the quality of the three bowel preparations.

Lund et al. (1998) showed that preparation of the left side of the colon is equally good whether the enema is administered by nurses in the endoscopy department or by patients at home.

In the study by Osgard et al. (1998) 164 adults undergoing flexible sigmoidoscopy at an ambulatory clinic were randomized to receive one of three preparations: a single hyperphosphate enema 1 h before the procedure; a hyperphosphate enema given one and two hours before the procedure; or a hyperphosphate enema administered one and two hours before the procedure, preceded by a 296 ml bottle of magnesium citrate taken p.o. the night before. All three preparations were equally well tolerated with slightly more diarrhoea reported among patients receiving magnesium citrate (p=0.007). The addition of magnesium citrate resulted in more procedures rated by the endoscopist as excellent or good (RR 1.5, 95 % CI: 1.3-1.9), deeper sigmoidoscope insertion (56 vs 51 cm, p=0.0036), fewer procedures requiring repeat preparation (RR: 0.21, 95 % CI: 0.04-0.98) and more procedures rated by patients as discomfort free (RR: 2.2, 95 % CI: 139-3.60). Excellent and good preparations were associated with shorter procedure duration (19 vs 14 min, p = 0.008) and greater depth of insertion (56 vs 50 cm, p=0.003).

In the study by Drew et al. (1997) 102 patients were randomized to receive either Picolax the evening before the examination (n= 46) or self-administered Fleet enemas (n= 56) prior to the investigation. Self-administered Fleet enemas provided a significantly superior bowel preparation with 52 (93%) being judged adequate or better, as opposed to 34 (74%) in the Picolax group. In addition, Fleet enemas were associated with significantly fewer adverse associated symptoms: 11 (20%) vs 24 (52%). Patients reported to be willing to receive Fleet enemas again in 53 (95%) vs 37 (80%) for the Picolax group.

Underwood et al. (2010) compared the efficacy and patient acceptability of two methods of bowel preparation for flexible sigmoidoscopy. Patients attending for outpatient flexible sigmoidoscopy were prospectively randomized to receive one Fleet ready-to-use enema or two times 4 g glycerine suppositories, two hours before the procedure. From November 2000 to August 2001, 203 patients were randomized. Endoscopist data available for 151 patients (enema = 76; suppository = 75) revealed: average depth of insertion (enema = 53.6 ± 11.6 cm; suppository 46.3 ± 13.7 cm; P < 0.001, Student's t test); acceptable (excellent + good) quality of preparation [enema = 60 (78.9%); suppository = 34 (45.3%); P < 0.0001, Fisher's exact]. The authors referred that bowel preparation for flexible sigmoidoscopy using a single Fleet enema is acceptable to patients and more effective than glycerine suppositories.

Observational study
Gross et al. (1955) examined the use of (self-administered) phosphate enemas immediately prior to examination. The authors concluded that the rectum and sigmoid could be rapidly cleansed and relaxed for sigmoidoscopy in a cancer detection centre by use of a phosphate enema.

Opinion statement
Nesselrod (1958) confirmed the importance of adequate bowel preparation prior to proctoscopy and recommended the use of Fleet Ready-to-Use Enema as a suitable bowel preparation agent.

Above publications support the clinical efficacy of sodium phosphate enemas for bowel cleansing prior to proctoscopy or sigmoidoscopy.

Aforementioned literature supports the use of sodium phosphate enemas before proctoscopy and sigmoidoscopy.

<u>Bowel cleansing before radiological examinations of the lower bowel</u>
A review (Skalnik 1978), open label studies (Jacobson & Thompson 2004, Yildar et al. 2017), and an observational study (Bevilacqua 1958) were submitted to substantiate the efficacy of bowel cleansing before radiological examinations of the lower bowel.

Review
Skalnik (1978) recommends the use of enemas with plastic disposable tips to prepare for bowel cleansing.

Open label studies
Jacobson & Thompson (2004) evaluated the changes of serum electrolytes over a two-hour period following the administration of a Fleet sodium phosphate enema (133 ml), or a Sodium phosphate enema Casen (250 ml) in healthy adults. In order to properly assess efficacy, subjects were asked to retain the enema fluid as long as possible, up to ten minutes. The time to bowel movements following the administration of the enema was recorded for each subject. An enema was considered effective if a bowel movement occurred after its administration. All subjects had at least one bowel movement, occurring between 30 seconds and ten minutes following the administration of the enema, thus, both enemas were considered effective. Mean retention times did not differ between the two enemas (p=0.9144), The mean retention time was 5.7 minutes in the 25 patients receiving Enema Casen, and of 6.0 minutes in the 20 patients receiving Fleet Enema.

Yildar et al. (2017) performed a prospective study on patients who were referred for elective total colonoscopy. Patients younger than 18 years of age or with previous colorectal resection were excluded. The standard oral purgative agent used in the pre-colonoscopy cleansing protocol contained sennoside A+B calcium (XM; solution 250 ml, Yenişehir Lab., Ankara, Turkey). The enema administered by the rectal route contained sodium hydrogen phosphate

and disodium hydrogen phosphate (BT; enema 210 ml, Yenişehir Lab., Ankara, Turkey). Patient satisfaction with the preparation procedure was 86.4% (196/227). Eighty (80) out of 151 patients were pre-treated with an enema administered by the rectal route contained sodium hydrogen phosphate and disodium hydrogen phosphate. No significant difference was determined in preparation procedure tolerance in terms of complications such as nausea, vomiting, abdominal pain, dizziness and headache. There was no significant difference between the groups in terms of total BBPS (Boston Bowel Preparation scale) scores (p=0.469). Right colon BBPS scores was increased with pre- purgative enema use, but the increase was not significant as compared to other groups (p=0.109).

Observational study
Bevilacqua (1958) conducted a radiological study in which he added barium to the Fleet Ready-to-use Enema and then administered this mixture to patients who were unselected and had not undergone bowel preparation. After five minutes, he took an abdominal X-ray with the patient in the prone position and observed that by five minutes, the solution was frequently observed to be in the proximal transverse colon and on several occasions had reached the caecum.

Bevilacqua (1958) then completed a series of 600 patients requiring bowel preparation for barium enema (both hospitalized and ambulatory cases) and found that no repeat investigations were required because of inadequate preparation. The author concluded that Fleet Ready-to-use Enema is an effective bowel preparation agent for barium examination, and by reasonable extrapolation, preparation for surgery and the more modern colonoscopy examination.

Aforementioned literature supports to a limited extent the efficacy of sodium sulphate enemas for bowel cleansing before radiological examinations of the lower bowel. Scientific evidence of a single publication (published more than 60 years ago) is considered limited to support the current efficacy of sodium phosphate enemas for radiological examinations in general.

<u>Dosing recommendation</u>
For the preparation of medical and diagnostic procedures it is proposed to administer one sodium phosphate enema of 133 ml one to two hours prior to the procedure in adults.

The rational for proposed posology for this indication was initially unclear. At least one bowel movement occurred in all adult study patients after administration of either a Fleet sodium phosphate enema (133 ml; n= 20) or a Casen sodium phosphate enema (250 ml; n= 25) in submitted study by Jacobson et al. (2010). The chemical composition of proposed sodium phosphate enema is the same as the 133 ml Fleet sodium phosphate enema described in literature (see quality assessment). Hence, appropriate bowel cleansing will also be obtained upon administration of one 133 ml unit of proposed sodium phosphate enema for the preparation of medical and diagnostic procedures in adults.

According to submitted literature, appropriate bowel cleansing is obtained in case a sodium phosphate enema is administered one to two hours prior to endoscopic evaluation of the lower bowel (i.e. sigmoidoscopy, proctoscopy) (Gidwani et al. 2007, Osgard et al. 1998, Underwood et al. 2010). The pharmacodynamic action of sodium phosphate will occur irrespective of a subsequent planned medical or diagnostic procedure. Hence, a medical or diagnostic procedure may be conducted one to two hours after the administration of a sodium phosphate enema in adults.

A similar posology for bowel cleansing before medical and diagnostic procedures in adults is recommended in the SmPCs of several authorized sodium phosphate enemas within the European Union (Colex enema solution for rectal use (NL/H/0619/001), Cleenema read-to-use 21.4g/9.4 g enema (marketing authorization number Ireland: PA 2028/1/1)). Because of this, proposed posology for the preparation of medical and diagnostic procedures in adults is considered acceptable.

Proposed dosing recommendations for above indication are supported by submitted evidence on dosing recommendations and the posology for similar indications of authorized sodium phosphate medicinal products (Colex enema solution for rectal use (NL/H/0619/001).

IV.5 Clinical safety

IV.5.1 Adverse events

Organized by MedDRA System Organ Class the undesirable effects of sodium phosphate enemas are listed below using the following frequency classification: very common (≥ 1/10); common (≥ 1/100 to < 1/10); uncommon (≥ 1/1,000 to < 1/100), rare (≥ 1/10,000 to < 1/1,000), very rare (< 1/10,000), not known (cannot be estimated from the available data) (MHRA, 2016; eMC, 2019):

- Immune System Disorders:
  - Very rare: Hypersensitivity e.g. urticaria.
- Skin and subcutaneous tissue disorders
  - Very rare: blister, pruritus, stinging.
- Metabolism and nutrition disorders
  - Very rare: tetany, dehydration, hyperphosphataemia, hypocalcaemia, hypokalaemia, hypernatremia, metabolic acidosis.
- Gastrointestinal disorders:
  - Very rare: nausea, vomiting, abdominal pain, abdominal distension, diarrhea, gastrointestinal pain, anal discomfort and proctalgia.
- General disorders and administration site conditions:
  - Very rare: rectal irritation, pain, stinging, chills.
- Investigations
  - Very common: temporary blood phosphorus increased.

Except from a very common (incidence ≥ 1/10) temporary increase in blood phosphorus concentrations, all other reported adverse drug reactions including dehydration and abnormal electrolyte levels (e.g. hyperphosphataemia, hypocalcaemia, hypokalaemia, hypernatremia) are very rare (incidence < 1/10,000).

The primary side effects caused by sodium phosphate enemas are water and electrolyte disturbances both in adult and paediatric paitents. The primary risk factors for these adverse events are: extreme age (i.e. < 5 years, >65 years), debilitated patients and associated comorbidities (Mendoza et al. 2007) like inflammatory colonic diseases (Crohn's disease, ulcerative colitis)(Campisi et al. 1999; Parra-Blanco 2006), delayed intestinal transit (megacolon, obstruction), conditions with intestinal vascular abnormalities (ischemic colitis)(Markowitz et al. 2005), congestive heart failure, impaired renal function, (Hookey and Vanner 2004; Chan et al. 1997; Fine and Patterson 1997) concomitant drugs that affect kidney perfusion and electrolyte levels (diuretics, angiotensin-converting enzyme inhibitors and angiotensin receptor blockers), medication known to prolong the QT interval, and Hirschprung´s disease in children.

Renal toxicity and nephropathy
48,018,292 Sodium phosphate enemas were sold in the last decade. Within the last decade, 15 cases of renal toxicity (frequency = 0.000000312380957) and one specific case of nephropathy (frequency = 0.000000020825397) were reported. It is unclear whether reported cases were related to sodium phosphate exposure.

The incidence of acute phosphate nephropathy is unclear. However, due to the low reported number of cases of renal toxicity and nephropathy during the use of sodium phosphate enemas, it is reasonable to assume that the incidence of acute phosphate nephropathy upon use of sodium phosphate enemas is also low.

IV.5.2 Serious adverse events

Water and electrolyte disturbances resulting from hyperphosphataemia, hypocalcaemia, hypernatraemia, and metabolic acidosis have been reported as serious adverse events. These serious adverse events may occur because of the absorptive effect of enema components and because of their inadequate elimination in some cases, such as patients with chronic renal failure (Mendoza et al., 2007).

Adverse events of sodium phosphate enemas were observed in 1.3% of patients in a five-year retrospective analysis by Anderson et al. (2019). The most frequently reported adverse events were abdominal pain and vomiting (both 0.5%). The most frequently reported adverse events upon sodium phosphate enema treatment in a study by Hansen et al. (2011) were emesis, diarrhoea, and abdominal pain/cramping. Comparable proportions of patients experienced none adverse events (38.5% vs. 43.8%) and one up to three adverse events (10.4% vs. 7.3%) upon treatment with respectively sodium phosphate enemas and milk and molasses enemas (p= 0.43).

A transient hyperphosphataemia was also observed in submitted open label study by Peery et al. (2005) and the case report by Nir-Paz et al. (1999). In a study by Alami et al. (2015) in 100 elderly patients with faecal impaction and renal impairment, 6% of patients developed hyperphosphataemia and 2% developed hypocalcaemia after administration of 1-6 sodium phosphate enemas. Low e-GFR and high baseline phosphate levels were found to be significant risk factors for developing post-enema hyperphosphataemia. According to Alami et al. (2015), it is safe to use sodium phosphate enemas in an elderly inpatient population with renal impairment.

IV.5.3 Laboratory findings

The primary side effects caused by sodium phosphate enemas are water and electrolyte disturbances both in adult and paediatric patients. The primary risk factors for these adverse events are: extreme age, debilitated patients and associated comorbidities (Mendoza et al., 2007) like inflammatory colonic diseases (Crohn's disease, ulcerative colitis), (Campisi et al., 1999; Parra-Blanco, 2006) delayed intestinal transit (megacolon, obstruction), conditions with intestinal vascular abnormalities (ischemic colitis), (Markowitz et al., 2005) congestive heart failure, impaired renal function, (Hookey and Vanner, 2004; Chan et al., 1997; Fine and Patterson, 1997) concomitant drugs that affect kidney perfusion and electrolyte levels (diuretics, angiotensin-converting enzyme inhibitors and angiotensin receptor blockers), medication known to prolong the QT interval, and Hirschprung´s disease in children.

IV.5.4 Safety in special populations

Age

As regards age distribution of patients reported as experiencing side effects, it should be noted that most of them were in the extreme age groups (older than 65 years and younger than 5 years, it should therefore be inferred that extreme ages are associated with a greater frequency of side effects.

Comorbid conditions

Comorbid conditions were noted in 86% of cases, particularly including neurological, gastrointestinal and renal disorders. Such associated conditions could be related to the increased phosphate absorption shown in some clinical trials. Thus, high serum phosphorus levels were shown in patients with a longer enema retention time.

Pregnancy and lactation

As there is no relevant data available to evaluate the potential for fetal malformation or other fetotoxic effects when administered during pregnancy sodium phosphate enemas should only be used as directed by a physician at the time of delivery or postpartum.

As sodium phosphate may pass into the breast milk, it is advised that breast milk is expressed and discarded for at least 24 hours after receiving sodium phosphate enemas (UptoDate Sodium Phosphate, 2019; eMC, 2019; MHRA, 2016).

IV.5.5 Overdose

Hyperphosphataemia, hypocalcaemia, hypernatraemia, dehydration, hypokalaemia, hypovolemia, acidosis and tetany may occur in overdose or retention. Recovery from the toxic effects can normally be achieved by rehydration. Treatment of electrolyte imbalance may require immediate medical intervention with appropriate electrolyte and fluid replacement therapy. Some references previously cited have shown clinical situations of overdose (Marraffa et al., 2004; Knobel and Petchenko, 1996).

Submitted publications (UpToDate 2019, Casais et al. 2009, Forman et al. 1979) are in line with above section. The publication by Ainley et al. (2005) addresses the increased risk of electrolyte disturbances in patients with comorbidity, and who use particular medications (e.g. ACE inhibitors, diuretics).

IV.5.6 Safety related to drug-drug interactions and other interactions

Phosphate enemas must be used with caution in patients taking calcium channel blockers, diuretics, lithium treatment or other medications that might affect electrolyte levels as hyperphosphataemia, hypocalcaemia, hypokalaemia, hypernatraemic dehydration and acidosis may occur. No other sodium phosphate preparations including sodium phosphate oral solution or tablets should be given concomitantly. As hypernatraemia is associated with lower lithium levels, concomitant use of Cleenema Ready-to-Use Enema and lithium therapy could lead to a fall in serum lithium levels with a lessening of effectiveness. (eMC, 2019; Drugs.com, 2019*)*

In addition, in other recent sources of data (UptoDate Sodium Phosphate, 2019; Lexicomp, 2019), the following information has been retrieved, regarding generic potential interactions of sodium phosphate. These interactions are more likely to be detected for oral administration and not in rectal use as sodium phosphate enema.

- *ACE Inhibitors*: May enhance the nephrotoxic effect of Sodium Phosphates. Specifically, the risk of acute phosphate nephropathy may be enhanced. Management: Consider avoiding this combination by temporarily suspending treatment with ACEIs or seeking alternatives to oral sodium phosphate bowel preparation. If the combination cannot be avoided, maintain adequate hydration and monitor renal function closely.

- *Angiotensin II receptor blockers*: May enhance the nephrotoxic effect of Sodium Phosphates. Specifically, the risk of acute phosphate nephropathy may be enhanced. Management: Consider avoiding this combination by temporarily suspending treatment with ARBs or seeking alternatives to oral sodium phosphate bowel preparation. If the

combination cannot be avoided, maintain adequate hydration and monitor renal function closely.

- *Antiacids*: May decrease the absorption of Phosphate Supplements. Management: This applies only to oral phosphate administration. Administer oral phosphate supplements at least one hour before, or two hours after, antacid administration.

- *Burosumab*: Phosphate Supplements may enhance the adverse/toxic effect of Burosumab.

- *Calcium salts*: May decrease the absorption of Phosphate Supplements. Management: This applies only to oral phosphate and calcium administration. Administering oral phosphate supplements at least one hour before or two hours after administration of an oral calcium salt may minimize the significance of the interaction.

- *Diuretics*: May enhance the nephrotoxic effect of Sodium Phosphates. Specifically, the risk of acute phosphate nephropathy may be enhanced. Management: Consider avoiding this combination by temporarily suspending treatment with diuretics or seeking alternatives to oral sodium phosphate bowel preparation. If the combination cannot be avoided, hydrate adequately and monitor fluid and renal status.

- *Erdafitinib*: Serum Phosphate Level-Altering Agents may diminish the therapeutic effect of Erdafitinib. Management: Avoid coadministration of serum phosphate level-altering agents with erdafitinib before initial dose increase period based on serum phosphate levels (days 14 to 21).

- *Iron preparations*: May decrease the absorption of Phosphate Supplements. Management: Administer oral phosphate supplements as far apart from the administration of an oral iron preparation as possible to minimize the significance of this interaction. Exceptions: Ferric Carboxymaltose; Ferric Gluconate; Ferric Hydroxide Polymaltose Complex; Ferric Pyrophosphate Citrate; Ferumoxytol; Iron Dextran Complex; Iron Isomaltoside; Iron Sucrose.

- *Magnesium salts*: May decrease the serum concentration of Phosphate Supplements. Management: This applies only to oral phosphate and magnesium administration. Administer oral phosphate supplements at least one hour before, or two hours after, oral magnesium salt administration.

- *Multivitamins/minerals (with A, D, E, K vitamins, Folate, Iron)*: May decrease the serum concentration of Phosphate Supplements. Management: This applies only to oral phosphate and multivitamin administration. Administer oral phosphate supplements at least one hour before, or two hours after, administration of an iron containing multivitamin.

- *Nonsteroidal Anti-Inflammatory Agents*: Sodium Phosphates may enhance the nephrotoxic effect of Nonsteroidal Anti-Inflammatory Agents. Specifically, the risk of acute phosphate nephropathy may be enhanced. Management: Consider avoiding this combination by temporarily suspending treatment with NSAIDs or seeking alternatives to oral sodium phosphate bowel preparation. If the combination cannot be avoided, maintain adequate hydration and monitor renal function closely.

- *Sucralfate*: May decrease the absorption of Phosphate Supplements. Management: This applies only to oral phosphate administration. Administering oral phosphate supplements at least one hour before or two hours after administration of sucralfate may reduce the significance of the interaction.

- *Tricyclic antidepressants*: May enhance the adverse/toxic effect of Sodium Phosphates. Specifically, the risk of seizure and/or loss of consciousness may be increased in patients with significant sodium phosphate induced fluid/electrolyte abnormalities.

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 Cleenema.

Table 2. 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 national procedure concerns a well-established use application for Cleenema. For this authorisation, reference is made to literature. No new clinical studies were conducted. Risk management is adequately addressed. Altogether it is considered that efficacy of sodium dihydrogen phosphate dihydrate and sodium phosphate dodecahydrate for the cleansing of the rectum, the sigmoideum and the lower part of the colon descendens in case of occasional constipation and if needed, for the preparation of medical and diagnostic procedures has been established as the majority of studies in subjects showed statistically significant and clinically relevant results. Finally, it is considered that the safety issues that are identified are adequately addressed in the SmPC.

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. The test consisted of a pilot test with three participants, followed by two rounds with ten participants each. The questions covered the following areas sufficiently: traceability, comprehensibility and applicability. The results show that the PL meets the criteria for readability as set out in the Guideline on the readability of the label and package leaflet of medicinal products for human use.

VI. OVERALL CONCLUSION, BENEFIT/RISK ASSESSMENT AND RECOMMENDATION

Cleenema 139,1 mg/ml – 31,7 mg/ml, solution for rectal use has a proven chemical-pharmaceutical quality. Cleenema has an adequate efficacy and safety profile and is considered widely established.

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 concerned member states, on the basis of the data submitted, considered that essential similarity has been demonstrated for Cleenema with the reference product, and have therefore granted a marketing authorisation. The decentralised procedure was finalised with a positive outcome on 10 December 2021.

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
942507	MAH transfer from Baggerman FarmaNet N.V. to Casen Recordati S.L.	EPAR, SmPC	25-3-2022	Approval	--

LITERATURE REFERENCES

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