OPINION OF THE SCIENTIFIC PANEL ON DIETETIC PRODUCTS, NUTRITION AND ALLERGIES ON A REQUEST FROM THE COMMISSION RELATED TO THE TOLERABLE UPPER INTAKE LEVEL OF PHOSPHORUS

(REQUEST N° EFSA-Q-2003-018)

(ADOPTED ON 1 JULY 2005 BY WRITTEN PROCEDURE)

SUMMARY

Phosphorus as phosphate is an essential nutrient involved in many physiological processes, such as the cell's energy cycle, regulation of the whole body acid-base balance, as a component of the cell structure (as phospholipids), in cell regulation and signalling, and in the mineralisation of bones and teeth (as part of the hydroxyapatite).

Estimates of habitual dietary intakes in European countries are on average around 1000-1500 mg/day, ranging up to about 2600 mg/day. The contribution of food supplements to phosphorus intake is low.

Adverse effects of excessive phosphorus intake, such as hyperphosphatemia, leading to secondary hyperparathyroidism, skeletal deformations, bone loss, and/or ectopic calcification have been reported in animal studies. However, such effects were not observed in studies in humans, except in patients with end stage renal disease. Although in acute or short term loading studies an increase in serum parathyroid hormone (PTH) levels has been found, no significant changes could be demonstrated in longer term studies with dosages up to 3000 mg/day (for 6 weeks). In these studies no evidence was found for effects on markers of bone remodelling and the Panel does not consider these to be adverse effects. Similarly, the Panel found no convincing evidence to support suggestions that high phosphorus diets would aggravate the effects of a state of secondary hyperparathyroidism induced by inadequate calcium intakes, or an inadequate vitamin D status.

Gastrointestinal symptoms, such as osmotic diarrhoea, nausea and vomiting, have been seen in some healthy subjects taking phosphorus (phosphate) supplements with dosages >750 mg/day. The Panel considered that these are not a suitable basis to establish an upper level for phosphorus from all sources.

The Panel concludes that the available data are not sufficient to establish an upper level for phosphorus.

The available data indicate that normal healthy individuals can tolerate phosphorus (phosphate) intakes up to at least 3000 mg/day without adverse systemic effects. In some individuals, however, mild gastrointestinal symptoms have been reported if exposed to supplemental intakes >750 mg phosphorus per day. There is no evidence of adverse effects associated with the current dietary intakes of phosphorus in EU countries.

KEY WORDS

Phosphorus, tolerable upper intake level, food safety.

BACKGROUND

In 2002, the European Parliament and the Council adopted the Directive 2002/46/EC[1] related to food supplements containing vitamins and minerals.

In addition, and as announced in its White Paper on Food Safety, the Commission aims to put forward a proposal for harmonising legislation concerning the addition of vitamins and minerals to foods.

With a view to provide scientific support to the European Commission's legislative work in this field, the Scientific Committee on Food (SCF) issued, from October 2000 to April 2003, a series of opinions on tolerable upper intake levels of individual vitamins and minerals and safety factors in relation to their use in fortified foods and food supplements (available on the Internet at: http://europa.eu.int/comm/food/fs/sc/scf/out80_en.html).

1. *Directive 2002/46/EC of the European Parliament and of the Council on the approximation of the laws of the Member States relating to food supplements. OJ L 183. 12.7.2002, p. 51.*

The SCF opinions covered 22 out of the 29 nutrients, which were considered to be within their mandate for this task. The SCF did not have sufficient time to adopt opinions for the following vitamins and minerals: vitamin C, chloride, fluoride, iron, phosphorus, potassium and sodium. In addition, during the decision making process for the adoption of Directive 2000/46/EC on food supplements the Parliament requested that boron, nickel, silicon, vanadium and tin should be allowed to be used in food supplements. Therefore, the European Food Safety Authority is asked to provide scientific opinions on the remaining 12 vitamins and minerals in accordance with the present terms of reference.

TERMS OF REFERENCE

With respect to the outstanding 12 vitamins and minerals, the European Food Safety Authority is asked 1) to review the upper levels of daily intakes that are unlikely to pose a risk of adverse health effects; 2) to provide the basis for the establishment of safety factors, where necessary, which would ensure the safety of fortified foods and food supplements containing the aforementioned nutrients.

ASSESSMENT

1. INTRODUCTION

Phosphorus is an element of group 5 of the periodic table and has an atomic weight of 30.97. It is one of the most abundant elements on earth, most commonly found as the phosphate ion (PO_4^{3-}), with phosphorus in its pentavalent form. Phosphorus (as phosphate) is an essential dietary constituent, involved in numerous physiological processes, such as the cell's energy cycle (high-energy pyrophosphate bonds in adenosine triphosphate [ATP]), regulation of the whole body acid-base balance, as component of the cell structure (as phospholipids) and of nucleotides and nucleic acids in DNA and RNA, in cell regulation and signalling by phosphorylation of catalytic proteins and as second messenger (cAMP). Another important function is in the mineralization of bones and teeth (as part of the hydroxyapatite).

Reviews and safety evaluations for phosphorus are available from the Joint FAO/WHO Expert Committee on Food additives (JECFA, 1982), the Food and Nutrition Board of the Institute of Medicine (FNB, 1997), and the UK Expert Group on Vitamins and Minerals (EGVM, 2003). JECFA has used the nephrocalcinosis, induced by excessive phosphate intake in rats, as the critical effect to set a maximum tolerable daily intake (MTDI) of 70 mg/kg for phosphoric acid and phosphate salts. The FNB set an upper level for phosphorus of 4.0 g/day for adults, based upon a NOAEL which represents the extrapolation of the phosphorus intake to serum phosphorus concentration curve in adults up to the intake of phosphorus which would result in serum phosphorus levels of infants, which are considered to be safe for tissues with respect to metastatic mineralization. The UK Expert Group on Vitamins and Minerals used the gastrointestinal effects due to high supplemental phosphate intake, to establish a NOAEL of 750 mg/day for supplemental phosphorus.

2. NUTRITIONAL BACKGROUND, FUNCTION, METABOLISM AND INTAKE

2.1. Food levels and dietary intake

Phosphorus is widely found in foods as phosphates; especially foods rich in protein are usually high in phosphorus, such as dairy products (100-900 mg/100 g), meats (200 mg/100 g), fish (200 mg/100 g) and grain products (100-300 mg/100 g). The average intake from foods in adults is usually between 1000-2000 mg/day. Dietary phosphorus intakes in various European countries are given in Table 1.

Table 1. The daily intakes of phosphorus in some EU countries (mg/day)

	Population	N	Method	Supplements	Mean	97.5 percentile
Italy[a]	Household	2374	7-day record	+	1304	2076
Germany[b]	Individual (M) Individual (F)	862 1144	7-day record + food frequency record	- -	1488 1188	2517 1988
Netherlands[c]	Household	5958	2-day record	-	1480	2601
Sweden[d]	Individual (M) Individual (F)	1214	7-day record	- -	1570 1290	2517 1988
UK[e]	Individual (M) Individual (F)	656 803	7-day record	- -	1493 1112	2381 1763

[a] Turrini (INRAN)
[b] Heseker *et al* (1994) - values are median and 95 percentiles.
[c] Hulshof and Kruizinga (1999)
[d] Becker and Pearson (2002) - values are mean and 95 percentiles.
[e] Henderson *et al* (2003)

Phosphoric acid and phosphate salts are also added as food additives in processed foods and in soft drinks as acidity regulators and stabilizers.

In the US the contribution from phosphorus-containing food additives is estimated at 320 mg/day, i.e. 20-30% of the adult phosphorus intake (Calvo and Park, 1996). No specific data on the contribution of phosphorus-containing food additives to the total intake of phosphorus in the EU have been identified, but this is likely increasing due to the higher consumption of processed foods and carbonated soft drinks. Cola soft drinks contain between 120-200 mg/L phosphorus. Fruit flavoured soft drinks are mostly acidulated with citrate rather than phosphoric acid, and contain little or no phosphate.

Phosphorus is also present in drinking water, with a maximum allowed content of 2.2 mg/L (Council Directive 80/778/EEC).

Dietary supplements may also contain phosphorus (as phosphates), but dose levels are generally low. A survey by the Netherlands consumer organisation indicated low to moderate phosphorus levels in the commonly sold supplements, i.e. <400 mg/tablet (Gezondgids Consumentenbond, 2002). The phosphorus content in multivitamin supplements, sold in the UK, varies between 15 and 1100 mg per supplement dose (EGVM, 2003). The contribution of dietary supplements to the average population intakes in the UK was found to be zero or negligible for all age groups (EGVM, 2003). High-protein bars and other products marketed for “enhanced” athletic performance and muscle mass building may also contain high levels of phosphorus.

2.2. Nutritional requirements and recommendations

The phosphorus requirement has often been linked to the calcium requirement, allowing a Ca: P weight ratio of about 1. The SCF based their requirements on a molar basis (1:1) with the calcium requirements and established a Population Reference Intake (PRI) of 550 mg/day in adults (17+) (SCF, 1993). For young children (6 months up to 3 years) the PRI is 300 mg/day, increasing up to 775 mg/day in young males (11-17 years), and 625 mg/day in young females. For lactating women the PRI was set at 950 mg/day, allowing an incremental 400 mg/day for breast milk production (SCF, 1993). In other countries, such as The Netherlands, lower ratios have been used, i.e. between 0.5 and 1.0. More recently the D-A-CH (2000) established a recommended intake of 700 mg/day for adults (19+), and up to 1250 mg/day for adolescents (10-19 years). The FNB make similar recommendations based upon maintenance of the serum inorganic phosphate level (Pi) within the normal range (FNB, 1997). For the younger age groups, a factorial approach was used, taking into account phosphorus accretion in bone and (lean) tissues.

As the body can maintain a phosphate (and calcium) balance over a wide range of intakes and Ca:P ratios, this ratio is nowadays considered of limited value for evaluation of dietary adequacy. Only in infants and children under conditions of rapid growth such a ratio has some relevance to enable optimal growth.

In human milk the Ca:P ratio is 1.5:1 (w:w), and this is considered the optimal ratio for the infant. This ratio equals more or less the ratio in human bone mineral (Nordin, 1976). The infant formula and follow-on formula Directive in the EU states that the Ca:P ratio should be between 1.2 and 2.0.

2.3. Function, uptake and distribution

Net absorption from a mixed diet has been reported to vary between 55-70% in adults (Lemann 1996; Nordin, 1986) and between 65-90% in infants and children (Ziegler and Fomon, 1983). There is no evidence that, contrary to calcium, absorption efficiency varies with dietary intake. Phosphate absorption is greatest in jejunum and takes place by a saturable, active transport mechanism, facilitated by 1,25-dihydroxyvitamin D, as well as by passive diffusion (Chen *et al*, 1974).

Some forms of dietary phosphorus are less bioavailable, especially phosphorus present in phytic acid in the outer coatings of cereal grains. The actual bioavailability depends on the way these grain products are processed and the amount of residual phytate. Some dietary components, as well as colonic bacteria, contain phytase activity rendering phytate phosphorus more available.

The phosphorus content of the adult human body is about 700 g (as elemental phosphorus). About 85% of total phosphorus is present in the skeleton, the remainder in the soft tissues and in the extracellular fluids (*circa* 1%) (Lloyd and Johnson, 1988). Total body phosphorus content increases from 0.5% on a body weight basis in infancy up to 0.65-1.1% in adults (Fomon and Nelson, 1993). Total phosphorus concentration in whole blood is approximately 13 mmol/L (40 mg/dL). Approximately 70% is present as organic phosphates, such as in the phospholipids of red blood cells and in the plasma lipoproteins. The other 30% is present as inorganic phosphate, of which 15% is protein bound. About 50% of the inorganic phosphate is in the soluble divalent cation form (HPO_4^{2-}), the remaining as the monovalent anion (H_2PO_{4-}, 10%) and the trivalent cation (PO_4^{3-}, <0.01%), or as HPO_4^{2-}complexed with sodium, calcium and magnesium salts. These anion forms are interconvertible and effective buffers of blood pH and involved in regulation of the whole body acid-base balance.

The inorganic phosphate (Pi) fraction in the extracellular fluid is of critical importance and under endocrine control of parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D, secondary to the regulation of serum calcium concentrations. Serum Pi is less tightly controlled than serum calcium and varies throughout the day following a circadian rhythm with peaks in the early morning and the afternoon (Portale *et al*, 1989; Calvo *et al*, 1991). Serum Pi in normal adults varies between 0.97-1.45 mmol/L (3.0-4.5 mg/dL), and shows a slight increase with increasing phosphorus intakes (Heaney, 1996). Hyperphosphatemia, associated with clear clinical symptoms, has only been reported in patients with end-stage renal disease, i.e. when glomerular filtration rate (GFR) has decreased below 20% of the adult value (FNB, 1997). In males, but not females, serum Pi values decrease with increasing age. Serum Pi values in young infants are about two times higher than in adults (1.29-2.26 mmol/L), due to a lower GFR, and a consequently lower phosphate excretion capacity in the kidneys in the first months of life (Manz, 1992; Endres, 1996).

PTH secretion can be induced by low calcium and high phosphorus intake, and results in a decrease in serum Pi by increasing urinary phosphate excretion (Calvo *et al*, 1988, 1990 and 1996). This PTH effect is mediated through the production of the active vitamin D metabolite 1,25-dihydroxyvitamin D by an effect on the 1-α-hydroxylase activity in the kidney. Excretion of endogenous phosphorus is mainly through the kidneys. Phosphate is filtered at the glomerulus and 80-90% is reabsorbed in the proximal renal tubules. Until recently, tubular reabsorption was thought to be mainly controlled by PTH and 1,25-dihydroxyvitamin D, but more recent evidence suggests an important regulatory role for a novel group of circulating phosphaturic hormones, called phosphatonins, such as the fibroblast growth factor 23 (FGF-23) (Quarles, 2003). Pi transport in the proximal tubule is driven by a Na+-dependent Pi transporter protein, residing in the brush-border membrane. Most proximal tubular Pi reabsorption occurs via the type 2a (NPT2) co-transporter. Dietary phosphorus intake also has a direct effect on the renal Pi excretion rate. Feeding a high phosphorus diet results in down-regulation of the NTP2 co-transporter (Keusch *et al*, 1998), while a low phosphorus intake will increase the amount of NTP2 cotransporter, and thus increase Pi reabsorption (Tenenhouse, 1997).

Genetic defects in the proximal tubular NTP2 co-transporters can impair renal Pi reabsorption and result in hyperphosphaturia, such as in X-linked hypophosphatemia and hereditary hypophosphatemic rickets with hypercalciuria (Tenenhouse, 1997; Murer *et al*, 1999).

2.4. Deficiency

The development of a dietary-induced phosphorus deficiency is very unlikely due to the ubiquitous presence in foods. Hypophosphatemia can occur in patients and infants receiving poorly managed parenteral nutrition, and in patients suffering from liver disease, sepsis, antacid therapy with aluminium containing drugs, and in diabetic ketoacidosis. Symptoms include anorexia, anaemia, muscle weakness, bone pain, rickets, and ataxia (Lotz *et al*, 1968). Inadequate intake of calcium and phosphorus has been associated with pathogenesis of bone disease in newborn infants (Bishop, 1989).

3. HAZARD IDENTIFICATION

The inorganic phosphate fraction in the extracellular fluid is under endocrine control of the parathyroid-vitamin D axis. Excess phosphorus intake might result in hyperphosphatemia and a consequent increase in serum PTH level. Secondary hyperparathyroidism leads to increased bone resorption which might adversely affect bone mineral density and skeletal integrity, and result in ectopic calcification. Such phosphorus induced effects have been observed in animal studies, but not in humans, except in patients with end-stage renal disease. As long as the kidney function, i.e. renal capacity, is adequate the excess phosphate is excreted. In some supplementation studies using high phosphorus dosages, osmotic diarrhoea and mild gastrointestinal symptoms have been reported.

3.1. Adverse effects in animals

3.1.1. Acute toxicity

Histological and histochemical changes have been described in the kidneys of rats fed for 24 to 72 hours a diet containing 10% disodium acid phosphate (providing approximately 5 g/kg body weight/day equivalent to about 1200 mg elemental phosphorus/kg body weight/day elemental phosphorus) (Craig, 1957).

Ritskes-Hoitinga *et al* (1989) found marked kidney calcification and a rise in albumin concentration in urine in female rats fed for 28 days a diet containing 0.6% sodium hydrogen phosphate (approximately 300 mg phosphorus/kg body weight/day). It was concluded that dietary phosphorus-induced nephrocalcinosis is associated with impaired kidney function in rats.

3.1.2. Sub-chronic toxicity

Pathological effects in the parathyroid, kidneys and bones have been observed in mature male rats fed a diet containing an excessively high level of sodium orthophosphate (8% in the diet which is approximately 4 g/kg body weight/day, providing about 1 g/kg body weight/day elemental phosphorus or 38 mmol phosphorus/kg body weight/day) for 7 months or until the animals succumbed (Saxton and Ellis, 1941). Microscopic examinations of the tissues at the time of death revealed hypertrophy and hyperplasia of parathyroid cells. Calcium deposits were present in the tubules of the kidneys and other organs. The long bones of the animals appeared thickened and more fragile than those of control animals.

In a study with three groups of 12 rats an adequate absorption and utilisation of calcium, phosphorus and iron was found after feeding a control (P: 210 mg/kg body weight/day; Ca: 280 mg/kg body weight/day), a normal orthophosphate (P: 215 mg/kg body weight/day; Ca: 235 mg/kg body weight/day), and a high orthophosphate diet (P: 650 mg/kg body weight/day; Ca: 250 mg/kg body weight/day). The experiment was conducted in three stages, with experimental observations made when animals had consumed the test diets for 50, 60 and 150 days (Dymsza *et al,* 1959). No adverse physiological effects were observed clinically, at autopsy, or on histological examination. The authors concluded that at both high and normal levels of dietary phosphorus the calcium, phosphorus and iron absorption and utilisation were adequate.

Haut *et al* (1980) investigated phosphate-induced renal injury in uninephrectomised, partially nephrectomised and intact rats. Phosphorus was administered in the diet at levels of 0.5, 1.0 and 2% (approximately 250, 500 and 1000 mg/kg body weight/day, respectively) for 18 weeks. None of the animals on a normal phosphorus intake (250 mg/kg body weight/day) showed any abnormalities. Four of 6 intact animals on the 1 % phosphorus diet (500 mg/kg body weight/day) had normal kidney calcium concentrations (one animal showed histological alterations in the kidneys). In contrast, all but one of the partial and uninephrectomised animals on a 1 % phosphorus diet (500 mg/kg body weight/day) had increased kidney calcium concentrations; 5 of the six animals in the group exhibited histological changes in the kidney. It was concluded that as renal functional mass is reduced, the nephrotoxicity of phosphorus is greatly enhanced.

Pettifor *et al* (1984) fed young baboons with semisynthetic, vitamin D-containing diets differing in calcium and/or phosphorus content over a 16 month study period. Diets low in calcium alone (40 mg/100 g) or low in both calcium and phosphorus (90 mg/100 g) led to the development of radiologic rickets and histologic features of osteomalacia at both 8 and 16 months. The diet which was low in calcium but which had a normal phosphorus content (310 mg/100 g) was associated with histologic features of hyperparathyroidism at 16 months; such features did not develop in animals fed the low calcium, low phosphorus diet. Biochemically the low calcium, normal phosphorus diet was associated with a transient fall in serum calcium around 8 months and a more persistent elevation in serum phosphorus and alkaline phosphatase values during the latter half of the study. These biochemical changes were not seen in the baboons on the low calcium, low phosphorus diet.

Based upon an evaluation of the available data from short-term and long-term studies with phosphoric acid and inorganic phosphate salts in rats and other species, JECFA concluded that excessive dietary phosphorus might result in homeostatic adjustments and subsequent bone loss and calcification of soft tissues (JECFA, 1982). The lowest level of phosphorus that produced nephrocalcinosis in the rat (i.e. 1% P in the diet) was used as the basis to set a maximum tolerable daily intake (MTDI) of 70 mg/kg for phosphoric acid and phosphate salts. This value was endorsed by the SCF (1991).

3.1.3. Genotoxicity

No genotoxic effect of inorganic salts of phosphorus was identified (JECFA, 1982).

3.1.4. Reproductive toxicity

Long-term effects of dietary phosphoric acid in three generations of rats have been investigated (Bonting and Jansen, 1956). The animals received diets containing 1.4% and 0.75% phosphoric acid (equivalent to approximately 200 and 375 mg phosphorus/kg body weight/day) for 90 weeks. No harmful effects on growth or reproduction were observed, and also no significant differences were noted in haematological parameters in comparison with control animals. There was no acidosis, nor any change in calcium metabolism. The quality of these older studies would be considered limited by current standards.

JECFA reviewed the available data from studies in mice and rats and concluded that dosing with phosphoric acid and inorganic phosphate salts does not induce maternal toxicity or teratogenic effects. Maximum dose levels tested for the various inorganic phosphate salts varied between 130 and 410 mg phosphorus/kg bodyweight (JECFA, 1982).

3.2. Adverse effects in humans

3.2.1. Acute effects

Osmotic diarrhoea and other mild gastrointestinal effects, including dyspepsia, nausea and vomiting have been observed as side effects in some individuals participating in supplementation studies using higher supplemental dosages (between 750-2250 mg/day; total oral intakes up to 3008 mg phosphorus/day) (Bernstein and Newton, 1966; Bell *et al*, 1977; Broadus *et al*, 1983; Grimm *et al*, 2001, Brixen *et al*, 1992; Whybro *et al*, 1998). Details of these studies are summarized in Table 2.

3.2.2. Adjustment in calcium-regulating hormones and effect on calcium balance and skeletal mass

High phosphorus intake results in the post-absorptive state in an increase in the serum Pi fraction and a subsequent temporary decrease in the serum ionized calcium level. These temporary changes in serum calcium are likely due to the phosphorus induced effects on the serum PTH and 1,25 dihydroxyvitamin D (1,25-$(OH)_2$D) levels, as part of the normal homeostatic control of the serum calcium concentration.

Most of the studies, summarized in Table 2, are of relatively small size (number of subjects included) and of short duration (single dose up to treatment of maximum 6 weeks), except some studies in patient groups, such as hypercalciuria patients (maximum 5 years) (Bernstein and Newton, 1966), multiple myeloma patients and osteoporotic women (maximum 15 months) (Goldsmith *et al*, 1968 and 1976), and hyperparathyroidism patients (12 months) (Broadus *et al*, 1983).

Table 2. Short description of relevant studies on phosphorus supplementation

Study/Authors	Subjects	Dose/Duration (as phosphorus)	Findings
Bernstein and Newton, 1966	Patients with idiopathic hypercalciuria (16-69 yr); n=10	Treatment with 700-2000 mg/day between 4 months and 5 years	Urine Ca ↓; reduced urinary stone formation → Mild to moderate diarrhoea
Reiss *et al*, 1970	Healthy subjects (23-46 yr); n=5	Single dose of 1000 mg/day	60-125% increase in PTH within 60 min. PTH increase abolished after Ca-infusion
Goldsmith *et al*, 1968	Multiple myeloma patients (n=14)	Intravenous and/or oral treatment with 1000-2000 mg/day; up to 15 months	Reduction in bone pain and in urinary calcium excretion. No indication of extra-skeletal calcification; one case of dyspepsia reported
Goldsmith *et al*, 1976	Postmenopausal women with osteoporosis (63-75 yr); n=7	1000 mg/day for 3-15 months Total P-intake: 1696-2740 mg/day; Ca-intake: 616-1459 mg/day	Urine Ca ↓; serum P ↓; no effect on PTH; evidence for increased bone resorbing surface; skeletal mass not measured
Van den Berg *et al*, 1980	Patients with idiopathic hypercalciuria; n=11	2000 mg/day for 2 weeks	Small increase in S-PTH (within normal range) and small decrease in 1,25-$(OH)_2$D; no effect on serum Ca and P-levels → No side effects reported
Broadus *et al*, 1983	Hyperparathyroid patients (mean age 53 yr), n=10	1500 mg/day for 12 months	s-PTH ↑; s-1,25-$(OH)_2$D ↓; urinary Ca ↓ → Several patients reported transient loosening of bowel movement
Portale *et al*, 1986	Healthy men; n=6	3000 mg/day for 10 days, after depletion period on 500 mg/day (+ aluminium hydroxide)	s-1,25-$(OH)_2$D ↓ (-30%); after initial rise in s-Pi, no significant change
Heaney and Recker, 1987	Premenopausal women (43.1 ± 4.4 yr); n=8	Basal diet (1166 mg/day) + supplements (1114 mg /day): total intake 2280 mg/day) for 4 months	no evidence for bone remodelling as measured by radiocalcium kinetics and histomorphometry
Calvo and Heath, 1988	Healthy men (n=8) and women (n=8); 18-25 yr	8 days on test diet with 420 mg Ca and 1660 mg P, after 8 days on control diet (820 mg Ca; 930 mg P)	s-PTH ↑; s-Pi ↑; s-1,25$(OH)_2$D ↑; urine c-AMP ↑; urine Hydroxyproline ↑
Calvo *et al*, 1990	Healthy women (mean age 22 yr); n=15.	Basal diet with 800 mg Ca and 900 mg P; after 28 days 10 women switched to diet with 400 mg/day Ca and 1700 mg/day P for 28 days	s-PTH ↑; no change in s-1,25-$(OH)_2$D; no change in s-osteocalcin and in bone resorption markers
Brixen *et al*, 1992	Postmenopausal women (50-75 yr); n=79	750, 1500 and 2250 mg/day (19-21 subjects per dose) for 7 days; 4 months follow-up. P-containing tablets given on top of regular diet (not specified)	s-PTH ↑ at P-intakes >1500 mg/day; no changes in serum Ca and P; urine P/creatinin-ratio; S-osteocalcin ↑ at 1500 mg, but not 2250 mg dose. No evidence of bone remodelling → Nausea, vomiting and diarrhoea in 2/19 patients at 750 mg dose, 3/19 at 1500 mg dose; 7/20 at 2250 mg dose
Wybro *et al*, 1998	Healthy men (19-38 yr); study 1: n=10 study 2: n=12	Study 1: 1000 mg/day for 1 week; standard diet: 800 mg/day of Ca and P each. Study 2: escalating dose study with 0, 1000, 1500, 2000 mg/day for 1 week; Standard diet: 1000 mg/day Ca and P each	Urine P ↑; urine Ca ↓; no changes in s-Pi, osteocalcin, and urinary N-telopeptide excretion. S-PTH increased only in study 1 → Diarrhoea in 1 subject receiving 2000 mg/day (study 2)
Grimm *et al*, 2001	Healthy women (20-30 yr); n=10.	3008 mg/day P and 1995 mg/day Ca for 6 weeks (incl. 1700 mg P from basal diet	No significant changes in s-PTH, osteocalcin, s-creatinin, and bone resorption markers → Mild intestinal distress (diarrhoea)

Abbreviations: S: serum; ↓: decrease; ↑: increase.; Ca: calcium; P: phosphorus; PTH: parathyroid hormone; 1,25-(OH)2D: 1,25 dihydroxyvitamin D; c-AMP: cyclic adenosine monophosphate.

In nearly all studies phosphorus supplementation resulted in an increased phosphate excretion and decreased calcium excretion. Some, but not all, studies show an increase in serum PTH after acute or long-term exposure to phosphorus loading. The variability in PTH response might, at least in part, be explained by the circadian rhythm in PTH secretion, and, as a consequence, of differences in time of blood sampling between studies. Acute oral phosphorus loading with dosages of 1.0-1.5 g phosphorus

did not result in an increase in serum PTH in young adults (Calvo and Heath, 1988). However, the same authors showed that feeding a "high phosphorus, low calcium" diet (1700 mg and 400 mg phosphorus/day, respectively) for 28 days resulted in a persistent increase in PTH in young healthy women, concurrent with a decrease in the serum ionized calcium level, while no such changes were seen in a control group fed a basal diet containing 900 mg P and 800 mg Ca (Calvo, 1994).

In the study by Brixen *et al* (1992) the PTH increase was not significantly different between the two highest dose groups (i.e. +1500 and +2250 mg phosphorus/day, respectively), but 3 times higher as compared to the control and low dose supplementation group (+750 mg phosphorus/day). In the studies by Whybro *et al* (1998) and Grimm *et al* (2001), PTH levels remained essentially unchanged after supplementation with 1500-2250 mg phosphorus daily for 1 and 6 weeks, respectively.

Under normal conditions an increase in PTH would induce renal synthesis of the active vitamin D metabolite 1,25-$(OH)_2$D. Studies from Portale *et al* (1986 and 1987), however, showed that high phosphorus dose levels decreased serum 1,25-$(OH)_2$D levels due to suppressed renal synthesis. This effect was already reported in the study by van den Berg *et al* (1980) and Broadus *et al* (1983), but not found in the studies from Calvo *et al* (1990) and Brixen *et al* (1992). In the latter study serum 1,25-$(OH)_2$D tended to be lower at the highest dose level (2250 mg/day), but this change was not significant.

It has been suggested that postmenopausal women might be more sensitive to the modulating effects of phosphorus on serum 1,25-$(OH)_2$D levels, i.e. less stimulation of 1,25-$(OH)_2$D production on a low Ca: high P diet, as compared to younger, but actual data are inconclusive (Calvo and Park, 1996).

Variable effects after phosphorus supplementation have been reported for markers of bone resorption. In the study by Goldsmith *et al* (1976) a decrease in bone-forming surface and bone-resorbing surfaces was found in a group of postmenopausal women with osteoporosis, given a daily dose of 1 gram phosphorus (as inorganic phosphate) on top of their normal diet. In an earlier study from the same authors (Goldsmith *et al*, 1968) in multiple myeloma patients on radiation or drug therapy (cyclophosphamide or melphalan), and suffering from bone pain and urinary calcium losses, oral and/or intravenous treatment with phosphate supplements (1000-2000 mg/day phosphorus) reduced the hypercalciuria, even in absence of hypercalcemia. X-ray examination did not indicate extra-skeletal calcification. In one patient even recalcification of the cervical spine was noted after 9 months on the 2 g per day oral phosphorus dose.

In a short-term study by Calvo *et al* (1988) in a group of young men and women (8 days on a test diet containing 420 mg/day Ca and 1660 mg/day P) an increase in urinary hydroxyproline and c-AMP excretion was found, also suggestive of increased bone turnover. However, in a follow-up study by the same authors (Calvo and Heath, 1990) using similar dose levels, but for a longer period (28 days), urinary hydroxyproline excretion did not significantly change. Plasma osteocalcin, a sensitive and specific marker of osteoblastic activity, an indicator for bone formation, remained unchanged.

In a controlled metabolic balance study in premenopausal women (n=8) doubling of the basal phosphorus intake from 1166 mg/day up to 2310 mg/day, by giving an additional mixture of sodium and potassium phosphate supplement for at least 4 months, while maintaining the calcium and protein intake constant, no evidence for bone remodelling was found as measured by radiocalcium kinetics and histomorphometry (Heaney and Recker, 1987).

Also in the more recent studies using more specific, "state of the art" markers of bone resorption, such as the urinary N-telopeptide excretion, it was concluded that high dose phosphorus supplementation did not significantly affect bone turnover (Whybro *et al*, 1998; Grimm *et al*, 2001).

In a cross-sectional epidemiological study among 510 healthy Danish women, aged 45-58 years, a positive association was found between the dietary Ca:P ratio and bone mineral density, apparently related by the inverse relationship observed between the Ca:P ratio and serum 1,25-$(OH)_2$D levels (Brot *et al*, 1999). These associations were found within the normal physiologic range of the 1,25-$(OH)_2$D levels.

However, no clinical studies have linked high phosphorus intake, with or without adequate calcium intake, to lower bone mass, or higher rates of bone loss in humans.

3.2.2.1. Newborns, young infants and children

Hypocalcaemia has occasionally been observed in neonates fed infant formula based on cows' milk, and related to the higher phosphorus load of these formulae (Venkatamaran *et al*, 1985; Specker *et al*, 1991). Infant formula based on unmodified cows' milk protein used to have a relatively high phosphorus level as compared to human milk, but a Ca:P ratio of about 1.0 to 2.0 is requested in modern formulae (Dorea 1999; Fomon, 1993). Neonates and young infants have a lower renal excretion capacity and, as a consequence, higher serum Pi values than older infants and adults at comparable (relative) intakes. This favours skeletal mineralization, but too high levels might adversely effect bone accretion, and in severe cases lead to rickets, and hypocalcaemic tetany. Specker *et al* (1991) demonstrated a linear relationship between serum PTH and mean phosphorus intake (range 100-250 mg/day) in neonates from 1-6 days of age, but not at 7-14 days of age.

Consumption of soft drinks with added phosphoric acid has also been associated with hypocalcaemia in children (Mazarlegos-Ramos *et al*, 1995). In a group of 57 children, ages between 1.5 -10 years, with a low serum calcium level (<2.2 mmol/L), soft drink consumption was much higher (>1.5 L per day) as compared to an age-matched control group with normal serum calcium values. This might be related however to low calcium intakes, rather than soft drink consumption as such, but no data on calcium intake were provided.

3.2.2.2. Women

Comparative studies in 20-40 year old women with carbonated beverages (567 mL) containing phosphoric acid or citric acid as the acidulant, did not indicate an effect on urinary calcium excretion (Heaney and Rafferty, 2001).

3.2.3. Ectopic calcification

Ectopic calcification as a result of high dietary phosphorus intake, as has been observed in mice and rats with normal kidney functions before exposure, has not been reported in humans with an adequate renal function. This might occur however in patients with end-stage renal disease associated with a variety of syndromes and (malignant) conditions. However, in these conditions, the hyperphosphatemia is not a direct, but a secondary effect.

3.2.4. Interaction with mineral and trace element absorption

Bour *et al* (1984) reported that high intakes of polyphosphates could interfere with absorption of iron, copper and zinc. However, this was not confirmed in a study by Snedeker *et al* (1982) who found no significant effect on iron, copper and zinc balance in 9 adult males after feeding a high phosphorus diet (2383 mg daily), in combination with a moderate (780 mg) or high (2442 mg) calcium diet for 39 days.

There is also no evidence that phosphorus interferes with calcium absorption. Studies from Spencer *et al* (1965) and Heaney (2000) have shown that over a wide range of Ca:P ratios in the regular diet, i.e. between 0.18 and 1.88, this ratio does not determine calcium absorption efficiency.

4. DOSE-RESPONSE ASSESSMENT

4.1. Osmotic diarrhoea and other mild gastrointestinal effects

Mild gastrointestinal complaints were reported in some individuals in some, but not all supplementation studies, at supplemental phosphorus intakes ≥750 mg/day (see Table 2). The Panel did not consider this effect as a suitable critical endpoint for setting an upper level.

4.2. Effect on calcium regulating hormones, calcium balance and skeletal mass

An increase in phosphorus intake can induce an increase in serum PTH, as part of the normal homeostatic control to maintain serum calcium levels. This effect depends on the actual increase in serum Pi, and the subsequent (small) decrease in the serum ionized calcium level. PTH adjusts renal clearance of Pi and through this mechanism the phosphate balance is maintained. It is not clear at what dietary intake level of phosphorus PTH secretion is stimulated. Only in the first days of life a (linear) relationship exists between serum PTH and phosphorus intake in the normal nutritional range. After the renal excretion capacity has fully developed and remains intact, the excess absorbed phosphate is excreted and serum Pi levels are kept within the normal range, i.e. no hyperphosphatemia develops.

Acute oral phosphorus loading with dosages of 1.0-1.5 g phosphorus did not result in an increase in serum PTH in young adults (Calvo and Heath, 1988). Also in an earlier study in osteoporotic postmenopausal women a supplemental phosphorus dosage of 1000 mg (total intake up to 2740 mg/day) for 12 months did not affect fasting serum PTH (Goldsmith *et al*, 1976). Also in more recent chronic supplementation studies in young healthy men (Whybro, 1998), and women (Grimm, 2001), with total intakes up to 3000 mg phosphorus/day, no significant effect on changes in serum PTH could be demonstrated. However in a study with a similar group of postmenopausal women supplemental dosages >1500 mg phosphorus/day resulted in an increase in PTH levels (+35%) (Brixen, 1992). A normal phosphorus intake (1700 mg/day) in combination with a low calcium diet (400 mg/day) also resulted in a persistent increase in PTH, but is likely due to the low calcium content, rather than the relatively higher phosphorus content (Calvo *et al*, 1990).

The skeletal effects of carbonated beverage consumption, if any, as reported in a relatively small study in children (Mazarlegos-Ramos *et al*, 1995) might be due to milk displacement, i.e. a low calcium intake rather than an effect of phosphoric acid. Such a trend in the consumption of milk and soft drinks, i.e. a decrease in milk consumption with a concurrent increase in soft drink consumption, resulting in a lower calcium, but higher phosphorus intake, has been reported for the US (Anderson *et al*, 2001).

A comparative study in adult women showed no effect of phosphoric acid compared to citric acid as the acidulant in carbonated beverages on urinary calcium excretion (Heaney and Rafferty, 2001).

Ectopic calcification (e.g. nephrocalcinosis) and skeletal deformations and bone loss, as observed in animal studies with high phosphorus loads, have not been reported to occur in humans as long as the renal excretion capacity is not seriously compromised, such as in end-stage renal disease.

It should also be noted that standard diets for laboratory animals generally have a relatively high phosphorus and low calcium content (JECFA, 1982). It cannot be excluded therefore that the observed effects in some of the animal studies were associated with the relatively low calcium intakes, rather than the high phosphorus intake as such. Besides the sensitivity of the PTH-vitamin D axis to variations in calcium and phosphorus intake might be different between animals and humans.

The suppression of the renal 1,25-$(OH)_2$D synthesis by an increase in the serum Pi level (Portale, 1986) contributes to the already decreased intestinal phosphate absorption, and increased renal phosphate excretion. This effect may be mediated by phosphatonins, under conditions of high phosphorus intake, due to down-regulation of the NTP2 co-transporters (Blumsohn, 2004).

Decreased absorption and increased renal excretion therefore protect the human body against the development of chronic hyperphosphatemia under conditions of high phosphorus intake. The observed decrease in responsiveness of osteoclasts to PTH with increasing serum Pi levels likely explain why a (temporary) increase in serum PTH, if any, induced by a high phosphorus load does not result in an increased rate of bone resorption (remodelling), as compared to a low calcium intake which induces both an increased PTH and 1,25-$(OH)_2$D synthesis. The role of the phosphatonins in these processes remains to be established (Blumsohn, 2004).

The phosphorus-induced nephrocalcinosis as observed in rats (JECFA, 1982), and the upper boundary of the normal distribution curve for serum Pi in infants (FNB, 1997) have been used as critical endpoints to set a maximum tolerable daily intake level. Both approaches have their limitations however. The phosphorus-induced nephrocalcinosis in rats seems less relevant for humans. Also derivation of a hypothetical NOAEL based upon serum Pi distribution curves is doubtful as no actual adverse effects on bone mineralization have been observed. The Panel decides therefore not to use these endpoints to derive an upper level.

CONCLUSIONS AND RECOMMENDATIONS

1. DERIVATION OF A TOLERABLE UPPER INTAKE LEVEL (UL)

The Panel considered that the adverse gastrointestinal effects that have been observed in some individuals exposed to high supplemental dosages (>750 mg/day) are not a suitable basis to establish an upper level (UL) for phosphorus from all sources.

An UL cannot be established based on the effect of a high phosphorus intake on the activity of calcium regulating hormones, which the Panel considers not to be adverse in themselves, and which have no demonstrable effects on bone mineral density and skeletal mass.

The Panel therefore concludes that the available data are not sufficient to establish an UL for phosphorus.

2. RISK CHARACTERISATION

The available data indicate that normal healthy individuals can tolerate phosphorus intakes up to at least 3000 mg phosphorus per day without adverse systemic effects. In some individuals, however, mild gastrointestinal symptoms, such as osmotic diarrhoea, nausea and vomiting, have been reported if exposed to supplemental intakes >750 mg phosphorus per day.

Estimates of current intakes of phosphorus in European countries indicate total mean dietary and supplemental intakes around 1000-1500 mg phosphorus per day, with high (97.5 percentile) intakes up to around 2600 mg phosphorus per day. There is no evidence of adverse effects associated with the current intakes of phosphorus.

Observational data suggest that high phosphorus intakes might aggravate the effects of a state of secondary hyperparathyroidism in individuals with inadequate calcium intakes, or an inadequate vitamin D status, e.g. postmenopausal women. The Panel considers that these data are not sufficient to establish the occurrence of such effects.

3. REFERENCES

Anderson JJB, Sell ML, Garner SC, Calvo MS (2001). Phosphorus. In: Present Knowledge in Nutrition. 8th Ed. BA Bowman & Russell RM (eds). ILSI Press, Washington DC.

Becker W and Pearson M (2002). Dietary habits and nutrient intake in Sweden (in Swedish with English summary). National Food Administration, Uppsala.

Bernstein DS and Newton R (1966). The effect of oral sodium phosphate on the formation of renal calculi and on idiopathic hypercalcuria. Lancet 2: 1105-1107.

Bell RR, Draper HH, Tzeng DY, Shin HK, Schmidt GR (1977). Physiological responses of human adults to foods containing phosphate additives. J Nutr 197: 42-50.

Bishop N (1989). Bone disease in preterm infants. Arch Dis Child 64: 1403-1409.

Blumsohn A (2004). What have we learnt about the regulation of phosphate metabolism? Curr opin Nephrol Hypertens 13: 397-401.

Bonting SL and Jansen B C (1956). The effect of a prolonged intake of phosphoric acid and citric acid in rats. Voeding 17: 137.

Bour NJS, Soullier BA, Zemel MB (1984). Effect of level and form of phosphorus and level of calcium intake on zinc, iron and copper bioavailability in man. Nutr res 4: 371-379.

Brixen K, Nielsen HK, Charles P, Mosekilde L (1992). Effect of a short course of oral phosphate treatment on serum parathyroid hormone (I-84) and biochemical markers of bone turnover: a dose-response study. Calcified Tissues International 51: 276-281.

Broadus AE, Magee JS, Mallette LE, Horst RL, Lang R, Jensen PS, Gertner JM, Baron R (1983). A detailed evaluation of oral phosphate therapy in selected patients with primary hyperparathyroidism. J Clin Endocrin Metab 56: 953-961.

Brot C, Jorgensen N, Madsen OR, Jensen LB, Sorensen OH (1999). Relationships between bone mineral density, serum vitamin D metabolites and calcium:phosphorus intake in healthy perimenopausal women. J Intern Med 245: 509-516.

Calvo MS (1994). The effects of high phosphorus intake on calcium homeostasis. Adv Nutr Res 9: 183-207.

Calvo MS and Heath H 3rd (1988). Acute effects of oral phosphate-salt ingestion on serum phosphorus, serum ionized calcium, and parathyroid hormone in young adults. Am J Clin Nutr 47:1025-1029.

Calvo MS and Heath H 3rd (1990). Persistently elevated parathyroid hormone secretion and action in young women after four weeks of ingesting high phosphorus, low calcium diets. J Clin Endocrinol Metab 70: 1334-1340.

Calvo MS and Park YK (1996). Changing phosphorus content of the U.S. diet: potential for adverse effects on bone. J Nutr 126:1168S-1180S.

Calvo MS, Kumar R, Heath H 3rd (1988). Elevated secretion and action of serum parathyroid hormone in young adults consuming high phosphorus, low calcium diets assembled from common foods. J Clin Endocrinol Metab 66: 823-829.

Calvo MS, Eastell R, Offord KP, Bergstralh EJ, Burritt MF (1991). Circadian variation in ionized calcium and intact parathyroid hormone: evidence for sex differences in calcium homeostasis. J Clin Endocrinol Metab 72: 69-76.

Chen TC, Castillo L, Korycka-Dahl M, De Luca HF (1974). Role of vitamin D metabolites in phosphate transport of rat intestine. J Nutr 104: 1056-1060.