

SCIENTIFIC OPINION

Chromium(III)-, iron(II)- and selenium-humic acid/fulvic acid chelate and supplemented humifulvate added for nutritional purposes to food supplements¹

Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food

(Questions No EFSA-Q-2006-191, EFSA-Q-2006-192, EFSA-Q-2006-193, EFSA-Q-2006-194)

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PANEL MEMBERS

F. Aguilar, U.R. Charrondiere, B. Dusemund, P. Galtier, J. Gilbert, D.M. Gott, S. Grilli, R. Guertler, G.E.N. Kass, J. Koenig, C. Lambré, J-C. Larsen, J-C. Leblanc, A. Mortensen, D. Parent-Massin, I. Pratt, I.M.C.M. Rietjens, I. Stankovic, P. Tobback, T. Verguieva, R.A. Woutersen.

SUMMARY

Following a request from the European Commission to the European Food Safety Authority, the Scientific Panel on Food Additives and Nutrient Sources added to Food was asked to evaluate the safety and bioavailability of iron(II)-, chromium(III)- and selenium- humic acid/fulvic acid chelate and of supplemented (dotated) humifulvate.

The present opinion deals only with the safety and bioavailability of particular sources of chromium iron and selenium as such, or of a series of minerals (including potassium, magnesium, iron, zinc, manganese, copper, molybdenum and selenium) present in supplemented humifulvate intended for the general population, to be used in food supplements. The safety of iron, chromium and selenium and of the other minerals present in supplemented humifulvate in terms of amounts that may be consumed is outside the remit of this Panel.

¹ For citation purposes: Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food on chromium(III)-, iron(II)- and selenium- humic acid/fulvic acid chelate and supplemented humifulvate added for nutritional purposes to food supplements following a request from the European Commission. *The EFSA Journal* (2009) 1147, 1-36

No data demonstrating the bioavailability of iron, chromium, selenium or any of the other mineral from their humic acid/fulvic acid chelates were provided.

The petitioner indicates that when the free metal binding capacity of humic substances is saturated or contains a high concentration of a metal, then humic substances will transfer this metal to the protein type molecules that are able to bind it. The petitioner stated that if the free metal binding capacity is high, then humic substances will form complexes with metals that are free or attached to metalloproteins.

The Panel concludes that the bioavailability of iron, chromium, selenium and other minerals from the humic acid/fulvic acid chelates (HFCs) and supplemented humifulvate has not been demonstrated, and that the actual level of saturation of the HFCs and supplemented (dotated) humifulvate has also not been characterised. Thus in theory, the bioavailability of iron, chromium, selenium and other minerals from the HFC sources might be lower than that of other sources. It could also be assumed that the source may actually cause chelation of metals provided by other sources, resulting in reduced bioavailability of the metals. Therefore, the Panel concludes that the bioavailability of iron, chromium, selenium and other minerals from their humic acid/fulvic acid chelates might be limited or even absent, whereas the possibility that the source may reduce the bioavailability of the metals and nutrients from other sources in the diet cannot be excluded.

The petitioner also indicated that humic acids are not easily definable compounds. Their place of origin may be more characteristic than the simple qualitative chemical analysis or any other investigation. Therefore, the Panel concludes that the specifications provided may only relate to the material described in the present Opinion originating from peat found primarily along the northern shores of Lake Balaton in Hungary.

Exposure resulting from supplement use of 1 g iron(II)-humic acid/fulvic acid chelate (Fe(II)-HFC) will amount to 68 mg humic/fulvic acid and 7 mg iron(II) per day, amounting to 1.1 mg humic/fulvic acid/kg bw/day and 0.12 mg iron/kg bw/day for a 60 kg person. The amount of iron is below the guidance value of 17 mg/day for supplemental intake proposed by the Expert group on Vitamins and Minerals (EVM).

Exposure resulting from supplement use of 1 g chromium(III)-humic acid/fulvic acid chelate (Cr(III)-HFC) will amount to 97 mg humic/fulvic acid and 58 µg chromium per day, amounting to 1.6 mg humic/fulvic acid/kg bw/day and 1 µg chromium/kg bw/day for a 60 kg person. The amount of chromium is below the total daily intake of about 150 µg Cr(III)/kg bw/day (approximately 10 mg/person) which was indicated by the EVM to be a level that would be expected to be without adverse health effects and also below the level of 250 µg/day that the World Health Organisation (WHO) considered as the level of chromium supplementation that should not be exceeded.

Exposure resulting from supplement use of 1 g selenium-humic acid/fulvic acid chelate (Se-HFC) will amount to 97 mg humic/fulvic acid and 57 µg selenium/day, amounting to 1.6 mg humic/fulvic acid/kg bw/day and 0.95 µg selenium/kg bw/day for a 60 kg person. The amount of selenium is below the recommended EC Tolerable Upper Intake Level (UL) for selenium of 300 µg/day.

Exposure resulting from supplement use of supplemented humifulvate will amount to levels up to 330 mg supplemented humifulvate with levels of minerals depending on the actual levels of these ingredients present. Based on the specifications provided by the petitioner, it can be calculated that 330 mg supplemented humifulvate will provide a daily dose of 82.5 mg humifulvate, 43.6 mg potassium, 17.8 mg magnesium, 16.8 mg iron, 11.9 mg zinc, 3.6 mg

manganese, 2.4 mg copper, 0.21 mg molybdenum and 0.15 mg selenium. For a 60 kg person, these daily doses amount to 1.38 mg humifulvate/kg bw/day, 0.73 mg potassium/kg bw/day, 0.3 mg magnesium/kg bw/day, 0.28 mg iron/kg bw/day, 0.20 mg zinc/kg bw/day, 60 µg manganese/kg bw/day, 40 µg copper/kg bw/day, 3.5 µg molybdenum/kg bw/day and 2.5 µg selenium/kg bw/day, respectively.

The amount of potassium is significantly lower than the guidance level for supplemental potassium intake (3 700 mg/day) proposed by the EVM in 2003.

The amount of magnesium is below the Tolerable Upper Level of 250 mg magnesium/day established by the Scientific Committee for Food (SCF) for readily dissociable magnesium salts.

The amount of iron is just below the guidance value of 17 mg/day for supplemental intake proposed by the EVM.

The amount of zinc amounts to 47.6% of the Tolerable Upper Level for zinc from all sources of 25 mg/day recommended by the SCF. It can not be excluded that the UL for zinc might be exceeded by the use of supplemented humifulvate in addition to other sources from the diet. The amount of manganese is below the guidance level of 4 mg/day for supplemental intake proposed by the EVM.

The amount of copper amounts to 48% of the UL for copper from all sources of 5 mg/day derived by the SCF. It can not be excluded that the UL for copper might be exceeded by the use of supplemented humifulvate in addition to other sources from the diet. The amount of molybdenum is lower than the UL of approximately 600 µg/day from all sources set by the SCF. It can not be excluded that the UL for molybdenum might be exceeded by the use of supplemented humifulvate in addition to other sources from the diet.

The amount of selenium is below the recommended EC Tolerable Upper Intake Level for selenium from all sources of 300 µg/day. It is not expected that the anticipated selenium intake including the use of supplemented humifulvate at the high intake level would exceed the UL.

The Panel notes that the UL is exceeded at the mean total exposure (diet plus supplement) for iron, potassium and manganese, and at the 97.5th percentile for all minerals except magnesium, selenium and chromium.

Total exposure to humic acid/fulvic acid from all four sources may amount to 344.5 mg humic acid/fulvic acid chelate (HFC) per day (68 + 97 + 97 + 82.5 mg/day, from the iron, chromium, selenium and supplemented HFC, respectively) amounting to 5.7 mg HFC/kg bw/day for a 60 kg person.

Toxicological studies provided on the HFCs are limited. The petitioner provided data from one 28-day toxicity study investigating the toxicity of the HFC material. Adult rats were fed HFC at levels of 5, 15 or 50 mg/kg daily for 28 days, or 150 or 500 mg/kg daily for 3 weeks. The Panel derives from this study a NOAEL of 50 mg HFC/kg bw/day.

The petitioner also described results from a 6-month (180-day) repeated dose oral toxicity study of supplemented potassium humate powder in beagle dogs. The Panel derives a NOAEL for supplemented potassium humate powder of 15 mg/kg bw/day, equivalent to 7 mg HFC/kg bw/day.

The Panel concludes that the margin of safety, between the NOAEL from the 28-day toxicity study in rats and the estimated human exposure, amounts to only 8.8, and between the NOAEL from the 180-day beagle dog study and the estimated human exposure to 1.2.

Therefore, the Panel concludes that the submitted data are insufficient to demonstrate the safety of the proposed use and use levels of iron(II)- chromium(III)- and selenium humic acid/fulvic acid chelate and supplemented (dotated) humifultate.

Key words:

Food supplements, chromium (III), iron (II), selenium, humic acid/fulvic acid chelate, CAS Registry Number 75350-40-2.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The European Community legislation lists nutritional substances that may be used for nutritional purposes in certain categories of foods as sources of certain nutrients.

The Commission has received a request for the evaluation of chromium(III)-, iron(II)- and selenium-humic acid/fulvic acid chelate and supplemented (dotated) humifultate EU-® added for nutritional purposes to food supplements. The relevant Community legislative measure is:

- 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 supplements².

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to provide a scientific opinion, based on its consideration of the safety and bioavailability of iron(II)-, chromium(III)-, and selenium-humic acid/fulvic acid chelate and of supplemented (dotated) humifultate EU-® added for nutritional purposes in food supplements.

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² OJ L 183, 12.7.2002, p.51.

ASSESSMENT

1. Introduction

The present opinion deals only with the safety and bioavailability of particular sources of iron, chromium and selenium, and other minerals intended for the general population, to be used in food supplements. The safety of iron, chromium, selenium and other minerals (potassium, magnesium, zinc, manganese, copper and molybdenum) in terms of amounts that may be consumed is outside the remit of this Panel.

2. Technical data

2.1. Chemistry

Humic and fulvic acids are multi-substituted polyaromatic heterocyclic macromolecules that incorporate protocatechuic acid, vanillic acid, vanillin, resorcinol, ferulic acid, benzoic acid, and other cyclic polyphenols resulting from the degradation of the lignin in plant cell walls. Humic and fulvic acids are rich in carboxyl, hydroxyl, and carbonyl groups as well as in phenols, quinones and semiquinones (Bruneton, 1995; Bravo, 1998; Yoshino, 1998). Within each macromolecule, aromatic groups are linked by amino acids, amino sugars, peptides and other aliphatic carbon chains (Buffle, 1977).

Humic substances are negatively-charged metal complexing ligands. There are a number of sites where metal ions may bind to aromatic and aliphatic carboxyl and phenolic hydroxyl groups within the humic acid complex, allowing humic substances to act as an ion exchanger, releasing metal ions of low atomic mass and chelating heavier metals (Aiken *et al.*, 1985; Norden and Dabek-Zlotorynska, 1997; Wershaw, 1989; Gramss *et al.*, 1999). These properties are abolished by ether methylation or acetylation of reactive sites (Frimmel and Christman, 1988).

The hypothetical structure provided by the petitioner for a humic acid is shown in Figure 1.

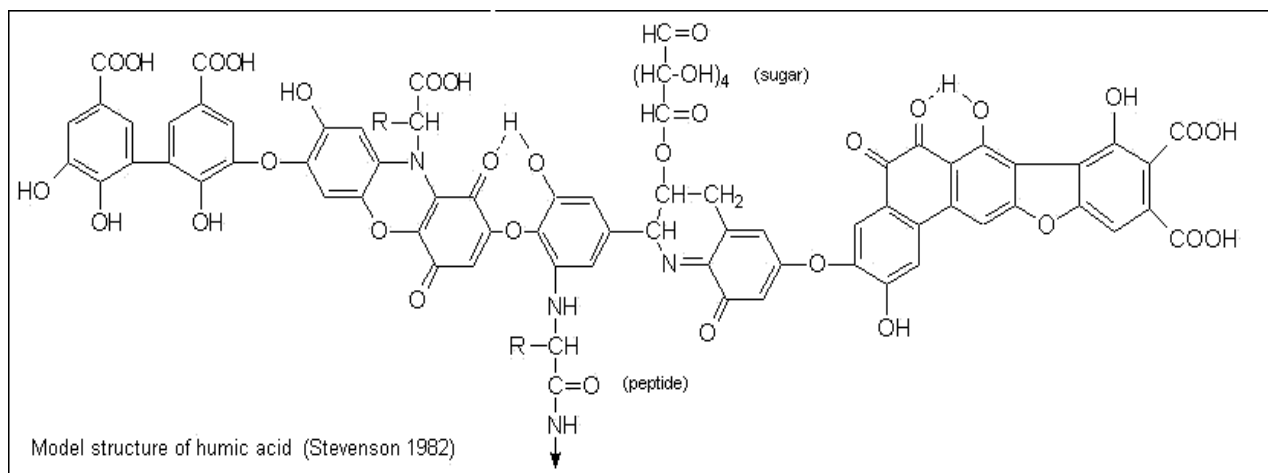


Figure 1. Structural formula of a humic acid (taken from Stevenson, 1982).

Due to the variable molecular composition of humic acids, a wide range of dissociation constants exists for the metals that are chelated by humic acids.

Fulvic acids are generally known to be more oxygen-rich and carbon-poor than humic acids, reflected by relatively higher number of carboxyl and phenolic functional groups. Similar to its humic acid counterpart, fulvic acid contains many reactive functional groups, including carbonyls, hydroxyls, phenols, quinones and semiquinones. These reactive groups make fulvic acid a candidate for both metal chelating and antioxidant activity. The molecular weights of fulvic acids are thought to be lower than those of humic acids. Figure 2 presents an example of a molecular structure of fulvic acid as provided by the petitioner.

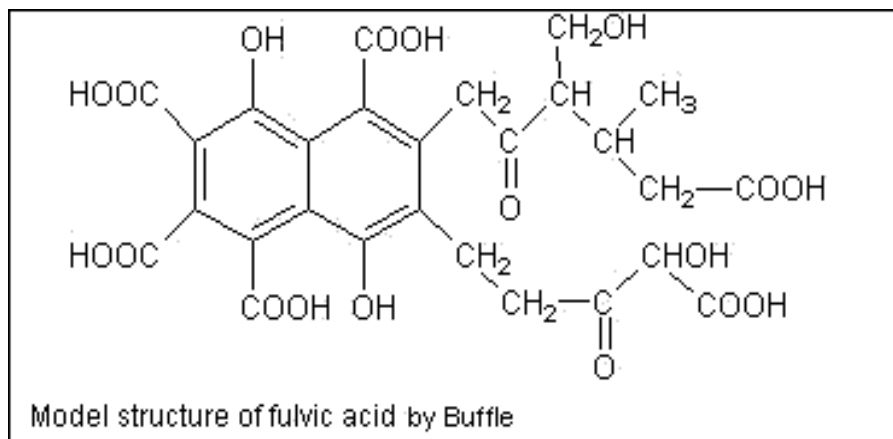


Figure 2. **Structural formula of fulvic acid (taken from Buffle, 1977).**

Due to the variable molecular composition of humic acids and fulvic acids, the molecular mass of humic/fulvic acids chelates are from as low as several hundreds to over 300,000 g/mol.

Iron(II)-humic acid/fulvic acid chelate (Fe(II)-HFC)

Iron(II)-humic acid/fulvic acid chelate (Fe(II)-HFC) is a brown coloured material in liquid or powder form. Solubility of the powder form in water is limited.

A synonym for iron(II)-humic acid/fulvic acid chelate is iron(II)-humat/fulvat.

Chromium(III)-humic acid/fulvic acid chelate (Cr(III)-HFC)

Chromium(III)-humic acid/fulvic acid chelate (Cr(III)-HFC) is a brown coloured material in liquid or powder form. Solubility of the powder form in water is limited.

A synonym for chromium(III)-humic acid/fulvic acid chelate is chromium(III) humat/fulvat.

Selenium-humic acid/fulvic acid chelate (Se-HFC)

Selenium-humic acid/fulvic acid chelate (Se-HFC) is a brown coloured material in liquid or powder form. Solubility of the powder form in water is limited.

A synonym for selenium-humic acid/fulvic acid chelate is selenium humat/fulvat.

Supplemented (dotated) humifulvate (supplemented HFC)

Supplemented humifulvate is a dark brown powder with characteristic odour. The petitioner indicates that the particle size is below 200 µm. The main constituent of supplemented humifulvate is humifulvate, which is a mixture of humic and fulvic acids of natural origin. Specific minerals are added to levels given below in the specification section.

The main humic acid constituent of humifulvate is humic acid with a molecular mass range of 5 000 - 100 000 g/mol. The amount of fulvic acid is rather low (<10 %). The petitioner indicates that the average molecular mass of fulvic acids is about 2000 g/mol and that the actual average molecular mass is dependent on the conditions: temperature, pH, ionic strength etc.

The petitioner indicates that 70-80 % of supplemented humifulvate is water soluble. The rest is non-soluble humic material as well as non-soluble humic/fulvic acid metal salts.

A synonym for supplemented humifulvate is dotated humifulvate. A CAS Registry Number is not available.

2.2. Specifications

Composition of the peat extract (humic substances)

The humic acid/fulvic acid material used for the chelates of the present opinion is extracted from peat derived from carboniferous plants applying a patented technology.

Table 1 and 2 present the specifications provided by the petitioner for the humic acid/fulvic acid chelates (HFCs).

Table 1. **Chemical composition of the humic acid/fulvic acid material present in chromium(III)-, iron(II)- and selenium-humic acid/fulvic acid chelate.**

Component	% of total weight
Organic substance	55-70
Carbon	20-39
Hydrogen	3-4
Nitrogen	2
Total protein content	10,5
Ash content	~ 30

Table 2. **Amino acid content in humic substances as provided by the petitioner.**

Amino acid	% of total	Amino acid	% of total
Aspartate	16.9	Isoleucine	5.2
Glutamate	13.1	Lysine	4.5
Glycine	10.4	Proline	3.9
Alanine	8.4	Arginine	3.3
Valine	7.8	Phenylalanine	2.9
Threonine	7.1	Histidine	2.0
Leucine	6.1	Methionine	1.9
Serine	5.2	Tyrosine	1.3

The petitioner also indicates that humic acids are not easily definable compounds and that their place of origin may be more characteristic than the simple qualitative chemical analysis or any other investigation. Therefore, the Panel notes that the specifications provided may only relate to the material described in the present Opinion originating from peat found primarily along the northern shores of Lake Balaton in Hungary.

Iron(II)-humic acid/fulvic acid chelate (Fe(II)-HFC)

The preparation of iron(II)-humic acid/fulvic acid chelate with additional iron(II) added, contains 95-97 g humic substances/100 g powder and 7.8-8 g iron (II)/100 g powder. Moisture is 1 - 3.5 g/100 g powder.

The purity of the humic substances is 80-90 %. The petitioner indicated the presence of the following impurities: residues of plants at 10-20 %, lead (Pb) at 4.5 mg/kg dry chelate material, arsenic (As) at 7.9 mg/kg, mercury (Hg) not detectable, and cadmium (Cd) at 0.5 mg/kg.

Chromium(III)-humic acid/fulvic acid chelate(Cr(III)-HFC)

The preparation of chromium(III)-humic acid/fulvic acid chelate with additional chromium(III) added, contains 96-98 g humic substances/100 g powder and 57 - 58 mg chromium(III)/100 g powder. Moisture is 1 - 3.5 g/100 g powder.

The purity of humic substances is 80-90 %. The petitioner indicated the presence of the following impurities: residues of plants at 10-20 %, lead (Pb) at 4.5 mg/kg dry chelate material, arsenic (As) at 7.9 mg/kg, mercury (Hg) not detectable, and cadmium (Cd) at 0.5 mg/kg.

Selenium-humic acid/fulvic acid chelate (Se-HFC)

The preparation of selenium-humic acid/fulvic acid chelate, with additional selenium added, contains 96-98 g humic substances/100 g powder and 57 - 58 mg selenium/100 g powder. Moisture is 1- 3.5 g/100 g powder.

The purity of humic substances is 80-90 %. The petitioner indicated the presence of the following impurities: residues of plants at 10-20 %, lead (Pb) at 4.5 mg/kg dry chelate material, arsenic (As) at 7.9 mg/kg, mercury (Hg) was not detectable, and cadmium (Cd) at 0.5 mg/kg.

Supplemented (dotated) humifulvate

The petitioner indicated that the constituents of the mixture and the proportion of each component are as follows: humifulvate 250,g/kg, potassium 132,g/kg, magnesium 54 g/kg, iron 51 g/kg, zinc 36 g/kg, manganese 11 g/kg, copper 7.25 mg/kg, molybdenum 0.63 g/kg, selenium 0.45 g/kg.

The petitioner also indicated that heavy metal contents of the material were as follows: lead \leq 0.17 g/kg, arsenic \leq 0.17 g/kg, aluminium \leq 3.33 g/kg.

The Panel notes that the specifications for lead are higher than what has been established in the Commission Regulation (EC) No 629/2008. The Panel notes that according to Commission Regulation (EC) No 629/2008 the maximum levels of lead, mercury and

cadmium, in food supplements as sold, should be 3.0 mg/kg, 0.1 mg/kg and 1 mg/kg, respectively.

2.3. Manufacturing process

The petitioner indicated that a peat by-product of the incomplete natural decomposition (humification) of organic plant material, humic substances, is derived from Hungarian peat found primarily along the northern shores of Lake Balaton in Hungary. Humic substances are processed into a concentrate for inclusion in dietary supplement in liquid and dried form intended for oral consumption.

To produce iron(II)-, chromium(III)- and selenium humic acid/fulvic acid chelate peat is extracted with a KOH solution of 1 % in filtrated water (temperature 50 °C) at an extract ratio of 1 kg/10 litres to obtain organic matter. The time of extraction is 16 hours (at 50 °C). Following a 24-hour settling down, the upper phase is separated into steel containers. The material is processed by ionization to obtain sufficient microbiological quality. The extract is concentrated by applying a membrane filtration process.

Iron(II)-humic acid/fulvic acid chelate (Fe(II)-HFC)

To prepare iron(II)-humic acid/fulvic acid chelate, 2.38 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ is added per kg concentrate and the material is dried by vacuum drying.

Chromium(III)-humic/fulvic acid chelate (Cr(III)-HFC)

To prepare chromium(III)-humic acid/fulvic acid chelate, 4 mg Cr(III) in the form of $\text{KCr}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ is added per kg concentrate and the material is dried by vacuum drying.

Selenium-humic acid/fulvic acid chelate (Se-HFC)

To prepare selenium-humic acid/fulvic acid chelate, 5.9 mg sodium-selenite is added per kg concentrate (amounting to 2.68 mg Se/kg concentrate) and the material is dried by vacuum drying.

Supplemented humifulvate

The petitioner states that basically humifulvate is extracted from special young peat, by means of 1% KOH solution. The extract is then sedimented and non-settled solid is then discarded. The supernatant colloidal mixture is supplemented by aqueous solutions of different metal salts and the resulted supplemented humifulvate colloidal solutions are spray-dried on very strict conditions. The manufacturing process involves addition of the following salts: K_2HPO_4 , MgSO_4 , ZnSO_4 , MnSO_4 , CuSO_4 , CoSO_4 , FeSO_4 , Na_2SeSO_3 , NaVO_3 , $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$. The resulted powder is ground, sieved and packed into double layer aluminum foil bags. The petitioner indicates that the material of the submission for supplemented humifulvate differs only from the cited procedure in the absence of added cobalt and vanadium.

2.4. Methods of analysis in food

The petitioner indicated that iron, chromium and selenium can be detected using Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES) methods, and that the humic acid/fulvic acid content can be determined only in the pure preparation using precipitation.

The petitioner for supplemented humifulvate indicated that the source is used exclusively as a food supplement and not used in food, and that therefore no analytical method was developed for its determination in foods. The petitioner also indicates that in principle, its amount can be determined in foods, on the basis of its metal-content by any known metal determination method.

2.5. Reaction and fate in foods to which the source is added

An interim report on the stability of the powder form of supplemented humifulvate was provided revealing that the powder is stable up to at least two years in storage.

In capsules containing supplemented humifulvate and 11.6 mg potassium, 5 mg magnesium, 4.67 mg iron, 3.33 mg zinc, 1 mg manganese, 0.667 mg copper, 0.167 mg vanadium, 0.067 mg cobalt, 0.058 molybdenum, 0.042 mg selenium, no signs of degradation were found during a 2-year observation period.

2.6. Case of need and proposed uses

Iron(II) -humic acid/fulvic acid chelate (Fe(II)-HFC)

The petitioner indicated that iron(II)-humic acid/fulvic acid chelate (Fe(II)-HFC) is to be used in pills containing 34 ± 2 mg humic/fulvic acid per pill and $3.5 \text{ mg} \pm 1 \text{ mg}$ iron(II) per pill, with a recommended dosage of 2 pills per day providing 68 mg humic/fulvic acid and 7 mg iron(II) per day, respectively.

Chromium (III)-humic acid/fulvic acid chelate (Cr(III)-HFC)

The petitioner indicated that chromium(III)-humic acid/fulvic acid chelate (Cr(III)-HFC) is to be used in pills containing 48.5 ± 2 mg humic/fulvic acid per pill and $29 \text{ } \mu\text{g} \pm 1 \text{ } \mu\text{g}$ chromium(III) per pill, with a recommended dosage of 2 pills per day providing 97 mg humic/fulvic acid and 58 μg chromium per day, respectively.

Selenium-humic acid/fulvic acid chelate (Se-HFC)

The petitioner indicated that selenium-humic acid/fulvic acid chelate (Se-HFC) is to be used in pills containing 48.5 ± 2 mg humic/fulvic acid per pill and $23.5 \text{ } \mu\text{g} \pm 2.5 \text{ } \mu\text{g}$ selenium per pill, with a recommended dosage of 2 pills per day providing 97 mg humic/fulvic acid and 57 μg selenium per day, respectively.

Supplemented (dotated) humifulvate

The petitioner indicated that supplemented humifulvate is used in different technological forms, i.e. capsules, tablets, effervescent tablets and syrup flavored with fruit juice with the

recommended daily dose being 330 mg or half or one third of this dose. Given that the supplemented humifulvate contains 250 g/kg humifulvate, the amount of 330 mg supplemented humifulvate per day provides a dose of 82.5 mg humifulvate per day.

2.7. Information on existing authorisations and evaluations

Iron(II)- chromium(III) and selenium-humic acid/fulvic acid

There are no existing authorisations or evaluations for these humic acid/fulvic acid chelates and also not for humic acid/fulvic acid.

Iron, chromium, selenium, zinc, manganese, copper, molybdenum, potassium and magnesium

Various opinions have evaluated the requirements and the safety of exposure to the minerals present in the various humic acid/fulvic acid chelate preparations of the present opinion and derived Tolerable Upper Intake Level (UL) values when possible. These data are given in Tables 3 to 5.

2.7.1. Iron

For iron, the Scientific Committee on Food (SCF) recommended daily intakes of 6 mg and 4 mg for infants aged 0.5-1 year and 1-3 years, respectively, assuming 15% absorption of the daily intake. For adults, assuming 10% absorption, the recommended dietary iron intake has been estimated to be between 8 and 10 mg iron/day in adult males, and 15 to 20 mg/day in adult females of reproductive age (SCF, 1993; IOM, 2001; D-A-CH, 2000).

Regarding upper limits, the Scientific Panel on Dietetic Products, Nutrition and Allergies of EFSA concluded in 2004 that the available data are insufficient to establish a Tolerable Upper Intake Level (UL) for iron. The opinion states that adverse gastrointestinal effects (e.g. nausea, epigastric discomfort, constipation) have been reported after short term oral dosage at 50-60 mg daily of supplemental non-haem iron preparations, particularly if taken without food. It was also concluded that an acute oral dose of 60 mg/kg bw can be lethal, but that oral doses below about 10-20 mg iron/kg bw do not cause acute systemic toxicity (EFSA, 2004). The US Food and Nutrition Board (FNB) established a UL of 45 mg/day for individuals aged 14 years and older, and 40 mg/day for younger age groups (IOM, 2001).

The Expert Group on Vitamins and Minerals (EVM) concluded that there were insufficient appropriate data to establish a Safe Upper Level for iron (EVM, 2003). It was also stated that for guidance purposes only, a supplemental intake of approximately 17 mg/day (equivalent to 0.28 mg/kg bw/day for a 60 kg adult) would not be expected to produce adverse effects in the majority of people.

2.7.2. Chromium

For chromium, the SCF stated that since data on essentiality and metabolism of chromium were so sparse, the Committee was not able to specify any requirements (SCF, 1993).

Recently, the Societies for Nutrition of Germany (DGE), Austria (ÖGE), and Switzerland (SGE) jointly established an adequate daily intake of 30-100 µg/day for adults (D-A-CH, 2000). In the US, the Food and Nutrition Board derived Adequate Intakes (AI) for chromium for different age groups, e.g. 35 µg/day and 25 µg/day for 19 to 50 year old men and women, respectively (IOM, 2001).

In 2003, the SCF was not able to derive a UL for chromium because available human data and the data from studies on subchronic, chronic and reproductive toxicity in experimental animals of soluble trivalent chromium salts did not provide clear information on the dose response relationships (SCF, 2003a). Also the US Food and Nutrition Board concluded that the data from animal and human studies were insufficient to establish a UL for soluble chromium (III) salts (IOM, 2001). The UK Expert Group on Vitamins and Minerals concluded that overall there are insufficient data from human and animals studies to derive a safe upper level for chromium. However, in the opinion of the EVM, a total daily intake of about 0.15 mg trivalent chromium per kg body weight per day (or 10 mg/person) would be expected to be without adverse health effects (EVM, 2003). WHO considered that supplementation of chromium should not exceed 250 µg/day (WHO, 1996).

2.7.3. Selenium

A Population Reference Intake (PRI) of 55 µg selenium/day for adults was established by the SCF in 1993 (SCF, 1993). The SCF has also established a UL for selenium of 300 µg/day (SCF, 2000a), while the EVM derived a Safe Upper Level (SUL) of 450 µg/day for total selenium (EVM, 2003). The US FNB estimated a UL of 400 µg/day (NAS, 2000). Both the SCF UL and that of the FNB also apply to pregnant and lactating women, and while the SCF commented that there were no specific data to support a derivation of a UL for children, they noted that there were no reports indicating that children were more susceptible than adults to adverse effects from selenium. Hence, they concluded that it was appropriate to extrapolate the UL from adults to children on a body weight basis (SCF, 2000a). This provided ULs ranging from 60 µg/day for children aged 1-3 years, 90 µg/day for children aged 4 - 6 years, and 250 µg/day for children aged 15 - 17 years. Specific legislative provisions on nutrient sources apply to foods manufactured for infants and young children.

2.7.4. Zinc

The European Population Reference Intake (PRI) for zinc, for adult males and females, is 9.5 mg/day and 7.0 mg/day, respectively (SCF, 1993). In the US, recent guidelines recommend daily intakes of 11 mg/day and 8 mg/day for adult men and women, respectively (IOM, 2001).

The SCF has established a UL for zinc, from all sources, of 25 mg/day (based on a NOAEL of 50 mg/day in humans for any adverse effects on a wide range of relevant indicators of copper status) (SCF, 2003b). The UK EVM established a SUL of 25 mg/day for supplemental zinc (EVM, 2003).

2.7.5. Magnesium

For magnesium, the SCF (1993) determined an Acceptable Range of Intake for adults of 150-500 mg/day.

The SCF (2001) established a UL of 250 mg magnesium/day for readily dissociable magnesium salts and compounds like magnesium oxide not including magnesium normally present in foods and beverages for adults and children from 4 years on.

2.7.6. Potassium

Recommended intakes for potassium are in the order of 3 100 - 3 500 mg/day (SCF, 1993).

The European Food Safety Authority has issued an opinion on Tolerable Upper Intake Levels of potassium (EFSA, 2005). It was concluded that the available data were insufficient to establish a UL.

The UK EVM concluded that there are insufficient data to establish a SUL for potassium. The amounts of potassium reported to cause adverse effects are variable and depend on factors which include formulation. However, they concluded that for guidance purposes, supplemental doses of up to 3 700 mg potassium/day appear to be without overt adverse effects (this is equivalent to 60 mg/kg bw/day in a 60 kg adult), but may be associated with gastrointestinal lesions diagnosed by endoscopy. Extrapolation of the guidance level to children on a bodyweight basis may be inappropriate (EVM, 2003).

2.7.7. Manganese

Currently there is no formal Recommended Dietary Allowance for manganese. The SCF estimated 1 - 10 mg/day as an acceptable range of intake (SCF, 1993).

The SCF concluded that they could not set a UL for manganese (SCF, 2000b). Also the UK EVM concluded that the data were insufficient to establish a SUL for manganese. They stated, however, that for guidance purposes it is reasonable that in the general population a supplemental intake of up to 4 mg manganese/day, in addition to the diet, would be unlikely to produce adverse effects (equivalent to 0.07 mg/kg bw for a 60 kg adult) (EVM, 2003).

2.7.8. Copper

An EU Population Reference Intake (PRI) of 1.1 mg for adults was established in 1992 (SCF, 1993).

The SCF has issued an opinion on Tolerable Upper Intake Levels of copper and derived a UL of 5 mg/day for adults and 1 - 4 g/day for children (SCF, 2003c).

2.7.9. Molybdenum

The SCF concluded that there are no reliable estimates of human requirements for molybdenum and no recommended intake has been established by the EC, other than that current intakes appear to be adequate and safe (SCF, 1993). More recently, IOM established a recommended daily intake of 45 µg/day (IOM, 2001) and the Societies for Nutrition of

Germany (DGE), Austria (ÖGE), and Switzerland (SGE), jointly established an adequate daily intake of 50 - 100 µg/day for adults (D-A-CH, 2000).

The SCF established a UL of 600 µg/day for adult. For children the UL, derived by extrapolation based on body weights, varied from 100 µg/day for young children to 500 µg/day for adolescents (SCF, 2000c).

2.8. Exposure

The petitioner indicated that humic and fulvic acids are not present in food.

Humic acids can be found in tap water as a result of biological and chemical conversion of plant material, mainly in water obtained from marsh soil.

2.8.1. Iron(II) -humic acid/fulvic acid chelate (Fe(II)-HFC)

The iron content of food varies greatly, and factors such as the soil, climate conditions and processing can influence the iron content of similar foods. The results can also be affected by differences in analytical methods. Foods rich in total iron include liver and offal, game and beef; cereals, cereal products and pulses also contain moderate to high levels. Poor sources of iron include milk and dairy products, whereas pork, poultry and green vegetables contain intermediate concentrations (EFSA, 2004).

The anticipated exposure to humic/fulvic acid, information on iron intake from food and supplements in European countries, the anticipated exposure to iron by using Iron (II)-humic acid/fulvic acid chelate, as proposed by the petitioner, and the Tolerable Upper Intake Level are summarised in Table 3. According to the SCF, the average and 97.5th percentile iron intakes from food in European countries vary from 10 to 17 mg/day and from 17 to 29 mg/day, respectively. The petitioner indicates that exposure resulting from supplement use will amount to 68 mg humic/fulvic acid and 7 mg iron(II)/day, amounting to 1.1 mg humic/fulvic acid/kg bw/day and 0.12 mg iron/kg bw/day for a 60 kg person.

The Panel noted that the anticipated exposure to iron through the use of iron (II)-humic/fulvic acid chelate as supplement, as indicated by the petitioner, would result in a total daily iron intake varying between 17 and 24 mg/day at the average level, and between 24 and 36 mg/day for high consumers not taking other iron-containing food supplements.

2.8.2. Chromium (III)-humic acid/fulvic acid chelate (Cr(III)-HFC)

Trivalent chromium occurs naturally in the food supply. The chromium content of foods is not included in existing food composition databases. Therefore, the intake can only be assessed using total diets or duplicate portion techniques.

The Total Diet Study (TDS) in the UK in 1997 reported that the mean chromium exposure was 100 µg/day and 170 µg/day at the 97.5th percentile, while the French TDS of 2001 indicated a mean exposure of 77 µg/day for adults > 15 years and 68 µg/day for children of 3 – 14 years, and at the 97.5th percentile of 126 µg/day for adults and 124 µg/day for children (Leblanc, 2005).

The petitioner indicates that exposure resulting from supplement use will amount to 97 mg humic/fulvic acid and 58 µg chromium/day, amounting to 1.6 mg humic/fulvic acid/kg bw/day and 1 µg chromium/kg bw/day for a 60 kg person.

The Panel noted that the anticipated exposure to chromium through use of chromium (III)-humic/fulvic acid chelate as supplement, as indicated by the petitioner, would result in a total daily chromium intake varying between 135 and 158 µg/day at the average level, and between 184 and 228 µg/day for high consumers not taking other chromium-containing food supplements (Table 3).

2.8.3. Selenium-humic acid/fulvic acid chelate (Se-HFC)

Selenium is a natural component of the diet and is present in plant foods such as cereals and nuts, but also in meat and seafood. The amount of selenium available in the soil for plant growth and corresponding variations in the intake of selenium by humans varies considerably among regions and countries (SCF, 2000a).

As shown in Table 3, the average and 97.5th percentile selenium intakes from food in European countries vary from 24 - 72 µg/day and 70 - 108 µg/day, respectively. The petitioner indicates that exposure resulting from supplement use will amount to 97 mg humic/fulvic acid and 57 µg selenium per day, amounting to 1.6 mg humic/fulvic acid/kg bw/day and 0.95 µg selenium/kg bw/day for a 60 kg person, respectively.

The Panel noted that the anticipated exposure to selenium through use of selenium-humic/fulvic acid chelate as supplement, as indicated by the petitioner, would result in a total daily selenium intake varying between 81 and 129 µg/day at the average level, and between 127 and 165 µg/day for high consumers not taking other selenium-containing food supplements (Table 3).

Table 3. Summary information on the intake of iron, chromium, and selenium, and anticipated exposure to humic/fulvic acid (HFC) from iron(II)-, chromium (III) and selenium- humic/fulvic acid chelate

	<i>HFC</i> (mg/day)	<i>Iron</i>			<i>Chromium</i>			<i>Selenium</i>		
		<i>Amount</i> (mg/day)	<i>Intake</i> (mg/day)		<i>Amount</i> (µg/day)	<i>Intake</i> (µg/day)		<i>Amount</i> (µg/day)	<i>Intake</i> (µg/day)	
			<i>Mean</i>	<i>P95 or P97.5</i>		<i>Mean</i>	<i>P95 or P97.5</i>		<i>Mean</i>	<i>P95 or P97.5</i>
Recommended Daily intake		m 8-10 f 15-20			m 35-100 f 25-100			55		
Maximum recommended or Tolerable Upper Intake level (UL)		17 S* GL**			10000 S* GL**			300		
Intake range from food in Europe			10-17	17-29		77-100	126-170		24-72	70-108
Humic acid/fulvic acid chelate Iron(II)-HFC	68	7			58					
Chromium (III)-HFC	97									

Selenium-HFC	97								57	
Total anticipated exposure from supplement, other uses and food intake			17-24	24-36		135-158	184-228		81-129	127-165

* S = for supplements

** GL = guidance value

2.8.4. Supplemented (dotated) humifulvate

The petitioner indicates that exposure resulting from supplement use will amount to levels up to 330 mg supplemented humifulvate with levels of minerals depending on the actual levels of these ingredients present. Based on the specifications provided by the petitioner, it can be calculated that 330 mg supplemented humifulvate will provide a daily dose of 82.5 mg humifulvate and 43.6 mg potassium, 17.8 mg magnesium, 16.8 mg iron, 11.9 mg zinc, 3.6 mg manganese, 2.4 mg copper, 0.21 mg molybdenum and 0.15 mg selenium. For a 60 kg person, these daily doses amount to 1.38 mg humifulvate/ kg bw/day, 0.73 mg potassium/kg bw/day, 0.3 mg magnesium/kg bw/day, 0.28 mg iron/kg bw/day, 0.2 mg zinc/kg bw/day, 60 µg manganese/kg bw/day, 40 µg copper/kg bw/day, 3.5 µg molybdenum/kg bw/day and 2.5 µg selenium/kg bw/day, respectively.

The anticipated exposure to humifulvate, information on potassium, magnesium, zinc, manganese, copper and molybdenum intake from food and supplements in European countries, the anticipated exposure to these micronutrients by using supplemented humifulvate as proposed by the petitioner, and the Tolerable Upper Intake Level are summarised in Tables 4 and 5.

2.8.4.1. Potassium

Important potassium sources include potatoes, fruit and berries, vegetables, milk products (excl. cheese) and nuts. Potassium occurs in foods mainly together with weak organic acids. Potassium is also found in mineral, spring, and table waters, but the content varies considerable (EFSA, 2005).

According to EFSA, the average and 95th to 97.5th percentile potassium intakes, from food in European countries, is in the range of 2 653 - 4 000 mg/day and 4 183 - 5 504 mg/day, respectively (Table 4).

The Panel noted that the anticipated exposure to potassium through use of supplemented humifulvate, as indicated by the petitioner, would result in a supplemental intake of approximately 44 mg/day, leading to total daily potassium intake varying between 2 697 and 4 044 mg/day at the average level, and between 4 227 and 5 548 mg/day for high consumers not taking other potassium-containing food supplements (Table 4).

2.8.4.2. Magnesium

Magnesium is ubiquitous in foods, but its content varies substantially. Leafy vegetables, as well as grains and nuts, generally have higher magnesium content (60-2700 mg/kg) than meat and dairy products (less than 280 mg/kg). Fats, refined sugars and pure alcohol are free of

Mg. Meat, most kinds of fish, fruit, most vegetables and dairy products contain less than 250 mg Mg/kg wet weight. Cacao and bitter chocolate, conches, shrimps, soybeans, butter beans and beet greens contain over 1000 mg Mg/kg. The Mg content of grain and grain products largely depends on processing, with high concentrations (1 100 - 1 800 mg/kg) found in whole barley, whole rye or wheat flour or brown rice (SCF, 2001; EVM, 2003).

According to the SCF (2001), the average and 97.5th percentile magnesium intakes from food in European countries vary from 208 to 353 mg/person/day and 350 to 628 mg/person/day, respectively (Table 4).

The Panel noted that the anticipated exposure to magnesium through use of supplemented humifulvate, as indicated by the petitioner, would result in total daily magnesium intake from supplements only of approximately 18 mg/day leading to a total daily magnesium intake of approximately 226 - 371 mg at the average level, and between 368 and 646 mg for high consumers not taking other magnesium-containing food supplements (Table 4).

2.8.4.3. Zinc

Zinc is widely distributed in foods. Good food sources of zinc include red meat, whole wheat, raisins and unrefined cereals (high zinc content, low availability). Milk, fruit and vegetables are low in zinc (Sandstead and Smith Jr, 1996).

According to the SCF (2003b), mean intakes of zinc in adults in Europe ranged from 7.5 to 12.1 mg/day. At the 97.5th percentile the highest intake values were 15 - 20.5 mg/day (Table 4).

The Panel noted that the anticipated exposure to zinc through use of supplemented humifulvate, as indicated by the petitioner, would result in a total daily zinc intake varying between 19.4 and 24 mg/day at the average level, and up to 26.9 - 32.4 mg/day for high consumers not taking other zinc-containing food supplements (Table 4).

Table 4. Summary information on the intake of potassium, magnesium and zinc, and anticipated exposure to (dotated) humifulvate

	<i>HFC</i> (mg/day)	<i>Potassium</i>			<i>Magnesium</i>			<i>Zinc</i>		
		<i>Amount</i> (mg/d)	<i>Mean</i>	<i>P95 or P97.5</i>	<i>Amount</i> (mg/d)	<i>Mean</i>	<i>P95 or P97.5</i>	<i>Amount</i> (mg/d)	<i>Mean</i>	<i>P95 or P97.5</i>
Recommended Daily Intake		3 100			150-500			M 9.5 F 7		
Maximum recommended or Tolerable Upper Intake level (UL)		3 700 S* GL**			250 S*			25		
Intake range from food in Europe			2653-4000	4183-5504		208-353	350-628		7.5-12.1	15-20.5
Supplemented (dotated) humifulvate	82.5	44			18			12		

Total anticipated exposure from supplement, other uses and food intake			2697-4044	4227-5548		226-371	368-646		19.4-24	26.9-32.4
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* S = for supplements

** GL = guidance value

2.8.4.4. Manganese

Manganese is present in foods, particularly green vegetables (2 mg/kg), nuts (14.9 mg/kg), bread (8 mg/kg) and other cereals (6.81 mg/kg). Tea is a rich source of manganese, containing 2.71 mg/kg and is the largest contributor to manganese intake (EVM, 2003).

According to total diet studies (MAFF, 1997; Leblanc, 2005), the average and 97.5th percentile manganese intakes from food in European countries is in the range of 2.3 to 4.9 mg/day and 4.8 to 8.2 mg/day, respectively (Table 5).

The Panel noted that the anticipated exposure to manganese through use of supplemented humifulvate, as indicated by the petitioner, would result in a supplemental intake of 3.6 mg/day and lead to a total daily manganese intake varying between 5.9 and 8.5 mg/day at the average level and between 8.4 and 11.8 mg/day for high consumers not taking other manganese-containing supplements (Table 5).

2.8.4.5. Copper

Food is the major source of copper intake, with particularly high concentrations being found in nuts (8 mg/kg), shellfish and offal (40 mg/kg). Other good sources are whole bran cereals and whole grain products (EVM, 2003; SCF, 2003c).

As shown in Table 5, mean dietary copper intakes from food of adults in different European countries have been estimated in the range of 1 - 2.2 mg/day at the average and 1.2 - 4.2 at the high level.

The Panel noted that the anticipated exposure to copper through use of supplemented humifulvate, as indicated by the petitioner, would result in a total daily copper intake varying between 3.4 and 4.6 mg/day at the average level and up to 6.6 mg/day for high consumers not taking other copper-containing food supplements (Table 5).

2.8.4.6. Molybdenum

Good sources of molybdenum are sorghum, vegetables (levels depending on soil content), legumes (beans), grains (cereals, wheat germ), organ meat (liver, kidney) milk and eggs. Some of 40% of molybdenum in cereals is lost on milling. Fruits, root vegetables and muscle meat are poor sources (SCF, 2000c).

Estimates of daily intakes vary widely regionally depending on the soil type. For adults, the representative range of mean estimates of molybdenum intakes in different countries is 80-250 µg/day on the average and 96 - 500 µg/day on the high level (SCF, 2000c) (Table 5).

The Panel noted that the anticipated exposure to molybdenum through use of supplemented humifulvate, as indicated by the petitioner, would result in a total daily molybdenum intake varying between 290 and 460 µg/day at the average level and between 306 and 710 µg/day for high consumers not taking other molybdenum-containing supplements (Table 5).

Table 5. Summary information on the intake of manganese, copper and molybdenum and anticipated exposure to (dotated) humifulvate

	HFC	Manganese			Copper			Molybdenum		
	(mg/day)	Intake (mg/day)			Intake (mg/day)			Intake(µg/day)		
		Amount	Mean	P95 or P97.5	Amount	Mean	P95 or P97.5	Amount	Mean	P95 or P97.5
Recommended Daily Intake		1-10			1.1			45-100		
Maximum recommended or Tolerable Upper Intake Level (UL)		4 S* GL**			5			600		
Intake range from food in Europe			2.3-4.9	4.8-8.2		1-2.2	1.2-4.2		80-250	96-500
Supplemented (dotated) humifulvate	82.5	3.6			2.4			210		
Total anticipated exposure from supplement, other uses and food intake			5.9-8.5	8.4-11.8		3.4-4.6	3.6-6.6		290-460	306-710

* S = for supplements
** GL = guidance value

2.8.4.7. Iron and selenium

As indicated by the petitioner, a dose of 330 mg supplemented humifulvate will provide a daily dose of 16.8 mg iron and 150 µg/day selenium. The Panel noted that given the reported intake (see Table 3) this would result in an anticipated total iron intake between 26.8 and 33.8 mg at the average, and between 33.8 and 45.8 mg/day for high consumers not taking other supplements. For selenium, the potential total mean intake would be between 174 and 222 µg/day and the high intake between 220 and 258 µg/day for consumers not taking other supplements.

2.8.5. Total exposure to humic acid/fulvic acid

Total exposure to humic acid/fulvic acid from all four sources may amount to 344.5 mg humic acid/fulvic acid chelate (HFC) per day (68 + 97 + 97 + 82.5 mg/day from respectively the iron, chromium, selenium and supplemented HFC) amounting to 5.7 mg HFC/kg bw/day for a 60 kg person.

3. Biological and toxicological data

3.1. Bioavailability

No data on the bioavailability of iron, chromium or selenium or other minerals from their humic acid/fulvic acid chelates were provided.

Humic substances have been reported to have the ability to transfer metals to and from metalloproteins *in vivo* (Glynn, 1995). The petitioner indicates that humic/fulvic acids have shown the ability to support the normal transport, absorption and distribution of essential nutrients and minerals in the body.

The petitioner also indicates that when the free metal binding capacity of humic substances is saturated or contains a high concentration of a metal, then humic substances will transfer this metal to the protein type molecules that are able to bind it. The petitioner states that on the other hand, if the free metal binding capacity is high then humic substances will form complexes with metals that are free or attached to metalloproteins.

The petitioner stated that research indicates that humic acid is absorbed *in vivo* (Frimmel and Christman, 1988; Visser, 1987; Lind and Glynn, 1999; Lehninger *et al.*, 1993).

One of the petitioners indicated that there are only a few studies on the absorption of humic/fulvic acid, but that the results are contradictory: in one of the experiments, fully labelled artificial “humic acid was administered and about 2/3 of the labelling appeared in the body. However, the molecular weight of the administered material was rather low, and the petitioner indicated that the material can rather be regarded as an artificially made low molecular weight fulvic acid. In contrast, upon administration of a “true”, partly labelled humic acid, less than 0.1 % of the material appeared in the body of experimental animals, so practically the material remained non-absorbed.

The petitioner described a series of experiments each performed with three male albino rats, weighing approximately 250 g. In one experiment the drinking water contained 1% of ¹⁴C-labelled humic acids (0.005 Ci/g). In another experiment 2 ml of a 1% solution of the ¹⁴C-labelled humic acids had been administered in a single dose to each of the rats by means of a stomach tube, and in a third experiment rats were used from which the bile duct had been cannulated and which had been injected i.p. with 0.5 ml of a 1% solution of ¹⁴C-labelled humic acids in saline (pH 7.0). At the end of each experiment, the ¹⁴C content of fluids such as blood, urine and bile was estimated, and ¹⁴C in the CO₂ of the air respired by rats as well as the ¹⁴C content of their faeces, urine and some of their internal organs was determined. From these results it became clear that humic acids can, at least partially, be absorbed by the gastrointestinal tract of the rat. From the formation of ¹⁴CO₂, from ¹⁴C-universally-labelled humic acids, it is shown that in the body of the rat a substantial fraction of the humic acid molecule can rapidly be metabolised.

Humic acids have been found to bind iodine to form iodo- derivatives, which may modify the biochemical behaviour of iodine resulting in a reduced uptake in animals and possibly in man as well (Hurrell, 1997; Fordyce *et al.*, 2000).

The Panel concludes that the bioavailability of iron, chromium, selenium and other minerals, from the humic acid/fulvic acid chelates (HFCs) and supplemented (dotated) humifultate, has not been demonstrated and that the actual level of saturation of the humic acid/fulvic acid chelates (HFCs) and supplemented (dotated) humifultate has also not been characterised. Thus in theory, the bioavailability of iron, chromium, selenium and other minerals from the HFC sources might be lower than that of other sources. It could also be assumed that the

source may actually cause chelation of metals provided by others sources, resulting in reduced bioavailability of the metals. Therefore the Panel concludes that the bioavailability of iron, chromium, selenium or other minerals from their humic acid/fulvic acid chelates might be limited or even absent, whereas the possibility that the source may reduce the bioavailability of the metals and nutrients from other sources in the diet cannot be excluded.

3.2. Toxicological Studies

The petitioners present short and long term toxicological animal studies as well as human data. These are described below.

The petitioner for supplemented humifulvic acid indicated that the formula tested in these studies refers to humifulvate plus added cations with the following composition: Humifulvate 75 mg (47.86%), total added minerals (as ions) 81.7 mg (52.14%), potassium (K_2HPO_4) 36.7 mg, magnesium ($MgSO_4 \times 7.H_2O$) 15 mg, iron ($FeSO_4 \times 7.H_2O$) 14 mg, zinc ($ZnSO_4 \times 7.H_2O$) 10 mg, manganese ($MnSO_4 \times H_2O$) 3 mg, copper ($CuSO_4 \times 5. H_2O$) 2 mg, vanadium ($NaVO_3$) 500 μ g, cobalt ($CoSO_4 \times 7.H_2O$) 200 μ g, molybdenum ($(NH_4)_6Mo_7O_{24}$) 175 μ g and selenium (Na_2SeO_3) 125 μ g, and was defined as (HFC). Where pure humifulvate was used, this was indicated. The Panel noted that in contrast to the specifications and the manufacturing procedure, vanadium and cobalt were present in the material for the toxicological studies.

3.2.1. Acute toxicity

The petitioner indicated that no signs of toxicity, gross organ pathology or death have been reported in single dose toxicity tests in male and female adult rats given up to 10 000 mg of standardized humic/fulvic acid chelate (HFC)/ per kg bw (equivalent to up to 4786 mg/kg of humifulvate). The petitioner indicated that acute studies in rats and mice have revealed no toxicity in daily doses exceeding 1,000 mg HFC/kg bw (equivalent to 478.6 mg/kg of humifulvate). The petitioner reported that adult male and female C57BL/6 mice, given 600 mg/kg of HFC (providing 287.3 mg/kg of humifulvate) within 24 hours, exhibited no signs of weight loss or macroscopic organ pathology (Kovacs, 1996). No symptoms of toxicity or lethality were observed during 14 days of post-treatment observation.

An evaluation of humic acids by EMEA (EMEA, 1998) states that humic acids are of low toxicity after oral administration and that the LD_{50} in rats is greater than 11 500 mg/kg bw.

3.2.2. Sub-chronic toxicity

The petitioner reported results from several semi-chronic toxicity studies and indicated that OECD guidelines were considered.

Adult rats, fed HFC at 5, 15 or 50 mg/kg bw daily for 28 days, exhibited no effects of HFC on body weights, clinical chemistry, haematological variables, enzyme functions or organ weights (Gachalyi *et al.*, 1994). However, three weeks of HFC at 150 or 500 mg/kg bw daily was associated with decreases in body weights and in liver and kidney weights, which the authors attributed to undocumented reductions in appetite. Since it was not investigated to what extent the effects on liver and kidney weight were accompanied by histological changes, the Panel concludes that the NOAEL from this study, as performed, is 50 mg/kg bw/day.

The petitioner also reported a study in which groups of adult rats fed potassium humate, providing either 60 or 240 mg/day of humic acid, for 2, 4, 6 or 8 weeks (amounting to approximately 300 or 1200 mg/kg bw/day assuming 200 g bw) exhibited growth rates, food consumption rates, physical agility, kidney and liver weights, white blood cell counts, red blood cell counts, thrombocyte counts, mean blood cell volumes, mean thrombocyte volumes, plasma hemoglobin concentrations, haematocrits, mean haemoglobin contents per red blood cell and mean red blood cell haemoglobin concentrations that were not different from those of control-fed rats. There were no adverse reactions, signs of toxicity or deaths during 8 weeks of exposure.

The petitioner also described results from a six-month (180-day) repeated dose oral toxicity study of supplemented potassium humate powder in beagle dogs. The study was performed according to GLP and to OECD guideline 409. The doses, chosen on the basis of the results of a preliminary study, were 0, 15, 50 and 150 mg/kg bw/day for 180 days (corresponding to daily doses of HFC of 0, 7, 24 and 71 mg/kg bw/day). Each of the four treatment groups consisted of four males and four females. The groups receiving placebo or 150 mg/kg bw/day also each included 2 male and 2 female “recovery animals.” The test item was applied daily (on a 7 days/week basis) by oral application, in gelatin capsule. Blood samples were taken from all animals receiving placebo or 150 mg/kg bw/day after one, three and six months. All animals were sacrificed after 6 months for detailed post-mortem examination.

There were no differences among the animals receiving 0 or 15 mg/kg bw/day (up to 3.5 times the recommended human intake) for 180 days. Vomiting and watery feces were occasionally associated with consumption of 50 mg/kg bw/day for the same period. Mild myocardial and hepatic lesions appeared in a few of the dogs receiving 150 mg/kg bw/day for 180 days.

The dogs consuming supplemental potassium humate powder exhibited significant increases in circulating molybdenum, selenium and vanadium concentrations, suggesting increased efficiency of absorption of these trace elements. There was no evidence of toxic accumulation of minerals or of accelerated excretory losses of required nutrients.

The petitioner indicated that a 180-day NOAEL for supplemented potassium humate powder of 15 mg/kg bw/day was observed. The petitioner indicated that this amount of supplemented potassium humate powder is equivalent to 7 mg HFC/kg bw/day.

An evaluation of humic acids by EMEA (EMEA, 1998) reports that in a 30-day toxicity study in rats, oral dose levels of 100 mg/kg bw/day of concentrated humic acid or of its sodium salt, had no effects on the behaviour and induced no clinical disturbances. It was also stated that the same results were obtained in dogs which received daily doses of 300 mg/kg bw for 90 days.

3.2.3. Reproductive and developmental toxicity

The petitioner described a study with adult rats fed a diet deficient in trace minerals and supplemented with either HFC at a daily dose equivalent to that recommended for humans or an equivalent amount of inorganic trace mineral salts, which exhibited no differences in average body weights, organ weights (liver, lung, kidney, brain, heart, spleen), total litter weights, individual birth weights of progeny, daily urine volumes and daily faecal weights. However, adult rats given humic acid at the equivalent of 280 times the recommended human

daily dose retained approximately 20 - 30% less dietary iron, zinc and selenium than did the rats that were fed HFC. Study details were not provided.

The EMEA evaluation of humic acid (EMEA, 1998) states that groups of 10 pregnant rats were treated orally with 5 000 sodium humate/kg bw/day on days 5 to 17 of pregnancy, or with 1 000 mg/kg bw/day on either days 5 to 9 or on days 11 to 15 of pregnancy. No teratogenic effects were seen.

3.2.4. Genotoxicity

The petitioner indicated that HFC has been found to exhibit no mutagenic activity under the Ames test criteria, using the *Salmonella typhimurium* reverse mutation assay, and that additional studies using human peripheral blood lymphocytes, also have indicated that HFC is not mutagenic and does not increase the number or frequency of chromosome aberrations (clastogenesis) under the test conditions applied. Study details were not provided.

The petitioner also reported that in a micronucleus test in bone marrow, ten NMRI mice (five males and five females per group) were given potassium humate powder at dose levels up to 2 000 mg/kg once via gastric intubation. The single administration of 2 000 mg/kg bw of the test item did not induce a statistically significant increase in the frequency of micronucleated polychromatic erythrocytes (MPCEs) in male and female mice at either 24 or 48 hours after treatment (compared to placebo). The investigators concluded that, under the conditions of this mouse micronucleus test, supplemented potassium humate powder in a single dose of 2,000 mg/kg was not mutagenic in NMRI mice. Study details were not provided.

3.2.5. Human studies

The petitioner indicated that the safety and tolerability profile of supplemented humifulvic acid was evaluated in human studies with a total of 1 125 patients. The treatment period of these studies varied from 14 days up to 72 months but information on the doses of a HFC containing syrup used were not provided. All the clinical reports and reported cases were evaluated for reported side- and adverse effects. The petitioner indicated that during the marketing period since 1994, serious side effects or adverse drug reactions were not reported either to the manufacturer or to the health authorities.

All these studies were not designed to investigate the safety of humic acid/fulvic acid.

3.2.6. Other studies

Various studies have reported biological effects of humic acid including, for example, elimination of heavy metals, desmutagenic effects (extracellular interception of mutagens) and antioxidant and anticoagulant activity (Shankel, 1993; Sato *et al.*, 1987; Cozzi *et al.*, 1993; Ferdinandy, 1997 (unpublished); Klocking, 1991; Riede *et al.*, 1991), immune-modulator effects (Baj *et al.*, 1993), increasing the product of Tumour Necrosis Factor alpha (TNF alpha) in human leukocytes and stimulating the synthesis of Interferon beta (IFN beta) (Inglot *et al.*, 1993), restituting the immune responses suppressed by zinc phosphamide in mice (Obminska-Domoradzka, 1993), decreasing the damage of gastric and duodenal mucosa in peptic ulcer (Brzozowsky, 1993), displaying regenerative effects in the liver (Malinski,

1993), inhibiting the reproduction of malignant tumour cells (Jurcsik, 1994) and use in treatment of certain forms of arthritis (Ruschev *et al.*, 1993).

Humic acid was stated to influence the function of the endocrine system. The effect on thyroid function was also studied in mice, and it was demonstrated that humic acids could antagonize the action of thyroxin (Huang, 1994).

All these studies were not designed to investigate the safety of humic acid/fulvic acid.

4. Discussion

The present opinion deals only with the safety and bioavailability of particular sources of iron, chromium, selenium and other minerals (potassium, magnesium, zinc, manganese, copper and molybdenum) intended for the general population, to be used in food supplements. The safety of iron, chromium, selenium and other minerals in terms of amounts that may be consumed is outside the remit of this Panel.

No data demonstrating the bioavailability of iron, chromium, selenium or other minerals from their humic acid/fulvic acid chelate sources were provided.

Humic substances have been reported to have the ability to transfer metals to and from metalloproteins *in vivo* (Glynn, 1995). The petitioner indicated that humic/fulvic acids have shown the ability to support the normal transport, absorption and distribution of essential nutrients and minerals in the body.

The petitioner also indicated that when the free metal binding capacity of humic substances is saturated, or contains a high concentration of a metal, then humic substances will transfer this metal to the protein type molecules that are able to bind it. The petitioner stated that on the other hand, if the free metal binding capacity is high then humic substances will form complexes with metals that are free or attached to metalloproteins.

The Panel concludes that the bioavailability of iron, chromium, selenium and other minerals from their humic acid/fulvic acid sources has not been demonstrated and that the actual level of saturation of the humic acid/fulvic acid has also not been characterised. Thus, in theory the bioavailability of iron, chromium, selenium and other minerals from the HFC sources might be lower than that of other sources. It could also be assumed that the source may actually cause chelation of metals provided by others sources, resulting in reduced bioavailability of the metals. Therefore the Panel concludes that the bioavailability of iron, chromium, selenium or other minerals from their humic acid/fulvic acid chelates has not been demonstrated and might even be limited or absent, whereas the possibility that the source may reduce the bioavailability of the metals and nutrients from other sources in the diet cannot be excluded.

Humic acids have been found to bind iodine to form iodo- derivatives, which may modify the biochemical behaviour of iodine resulting in a reduced uptake in animals and possibly in man as well.

The petitioner also indicated that humic acids are not easily definable compounds. Their place of origin may be more characteristic than the simple qualitative chemical analysis or any other investigation. The Panel therefore concludes that the specifications provided may only relate to the material described in the present Opinion, originating from peat found primarily along the northern shores of Lake Balaton in Hungary.

The European Food Safety Authority has issued an opinion on the Tolerable Upper Intake level of iron (EFSA, 2004). It was concluded that the available data were insufficient to establish a Tolerable Upper Intake Level for iron. The opinion states that adverse gastrointestinal effects (i.e. nausea, epigastric discomfort, constipation) have been reported after short term oral dosage at 50 - 60 mg daily of supplemental non-haem iron preparations, particularly taken without food. It was also concluded that an acute oral dose of 60 mg/kg bw can be lethal, but that oral doses below about 10-20 mg iron/kg bw do not cause acute systemic toxicity.

The Expert Group on Vitamins and Minerals (EVM, 2003) also concluded that there are insufficient data to establish a Safe Upper Level for iron. It was also stated that for guidance purposes, a supplemental intake of approximately 17 mg/day (equivalent to 0.28 mg/kg bw/day for a 60 kg adult) would not be expected to produce adverse effects in the majority of people.

Exposure resulting from supplement use of iron(II)-humic acid/fulvic acid will amount to 68 mg humic/fulvic acid and 7 mg iron(II) per day, amounting to 1.1 mg humic/fulvic acid/kg bw/day and 0.12 mg iron/kg bw/day for a 60 kg person. The amount of iron is below the guidance value for supplemental intake proposed by EVM (EVM, 2003).

Exposure resulting from supplement use of 1 g chromium(III)-humic acid/fulvic acid chelate (Cr(III)-HFC) will amount to 97 mg humic/fulvic acid and 58 µg chromium per day, amounting to 1.6 mg humic/fulvic acid/kg bw/day and 1 µg chromium/kg bw/day for a 60 kg person. The anticipated high level intake of chromium from the diet and the supplement (up to 228 µg/day equivalent to 3.8 µg/kg bw/day) is below the total daily intake of about 150 µg Cr(III)/kg bw/day (approximately 10 mg/person) which was indicated by the EVM to be a level that would be expected to be without adverse health effects. The amount provided by the supplement is also below the level of 250 µg/day that the WHO considered that supplementation of chromium should not exceed.

For the exposure assessment of the minerals it is assumed that the particular supplement under consideration is the only supplement source.

Exposure resulting from supplement use of 1.0 g selenium-humic acid/fulvic acid chelate (Se-HFC) will amount to 97 mg humic/fulvic acid and 57 µg selenium per day, amounting to 1.1 mg humic/fulvic acid/kg bw/day and 0.95 µg selenium/kg bw/day for a 60 kg person. The anticipated high level of selenium from the diet and the supplement (up to 165 µg/day) is below the recommended EC UL for selenium of 300 µg/day for the adult population.

Exposure resulting from supplement use of supplemented humifulvate will amount to levels up to 330 mg supplemented humifulvate with levels of minerals depending on the actual levels of these ingredients present. Based on the specifications provided by the petitioner, it can be calculated that 330 mg supplemented humifulvate will provide a daily dose of 82.5 mg humifulvate, 43.6 mg potassium, 17.8 mg magnesium, 16.8 mg iron, 11.9 mg zinc, 3.6 mg manganese, 2.4 mg copper, 0.21 mg molybdenum and 0.15 mg selenium. For a 60 kg person, these daily doses amount to 1.38 mg humifulvate/kg bw/day, 0.73 mg potassium/kg bw/day, 0.3 mg magnesium/kg bw/day, 0.28 mg iron/kg bw/day, 0.2 mg zinc/kg bw/day, 60 µg manganese/kg bw/day, 40 µg copper/kg bw/day, 3.5 µg molybdenum/kg bw/day and 2.5 µg selenium/kg bw/day, respectively.

The amount of potassium is significantly lower than the guidance level for supplemental potassium intake (3 700 mg/day) proposed by the EVM (2003).

The amount of magnesium is below the tolerable upper level of 250 mg magnesium/day established by the SCF for readily dissociable magnesium salts (SCF, 2001).

The amount of iron is just below the guidance value of 17 mg/day for supplemental intake proposed by the EVM (EVM, 2003).

The amount of zinc amounts to 47.6% of the UL for zinc from all sources of 25 mg/day recommended by the SCF (SCF, 2002b). Since the average intake of zinc of European adults ranged from 7.5 to 11.4 mg/day and the high level intake from 15 to 20.5 mg/day it cannot be excluded that the UL for zinc might be exceeded by the use of supplemented humifulvate in addition to other sources from the diet.

The amount of manganese is below the guidance level of 4 mg/day for supplemental intake proposed by the EVM (2003).

The amount of copper amounts to 48% of the Tolerable Upper Level for copper from all sources of 5 mg/day derived by SCF (SCF, 2003c). Since the intake of copper ranged from 1 to 2.2 mg/day at the average, and from 1.2 to 4.2 mg/day at the high level, it cannot be excluded that the UL for copper might be exceeded by the use of supplemented humifulvate in addition to other sources from the diet.

The amount of molybdenum is lower than the UL of approximately 600 µg/day from all sources set by the SCF (SCF, 2000c). Since the intake of molybdenum ranged from 80 to 250 µg/day at the average, and from 96 to 500 µg /day at the high level, it cannot be excluded that the UL for molybdenum might be exceeded by the use of supplemented humifulvate in addition to other sources from the diet.

The amount of selenium is below the recommended EC UL from all sources for selenium of 300 µg/day (2000a). At the high intake level the anticipated selenium intake including the use of supplemented humifulvate is not expected to exceed the UL.

The Panel notes that the UL is exceeded at the mean total exposure (diet plus supplement) for iron, potassium and manganese, and at the 97.5th percentile for all minerals except magnesium, selenium and chromium.

Total exposure to humic acid/fulvic acid from all four sources may amount to 344.5 mg humic acid/fulvic acid chelate (HFC)/day (68 + 97 + 97 + 82.5 mg/day from the iron, chromium, selenium and supplemented HFC, respectively) amounting to 5.7 mg HFC/kg bw/day for a 60 kg person.

The Panel noted that HFC was reported to be negative in various *in vitro* genotoxicity tests (*Salmonella typhimurium* reverse mutation assay; and a test for chromosome aberrations in human peripheral blood lymphocytes). HFC was also reported to be negative in an *in vivo* mouse micronucleus test in bone marrow although the Panel noted the absence of evidence for exposure of the target tissue (bone marrow) in this study.

The petitioner provided data from one 28-day toxicity study investigating the toxicity of the humic acid/fulvic acid chelate (HFC) material. Adult rats, fed HFC at 5, 15 or 50 mg/kg bw daily for 28 days, exhibited no effects of HFC on body weights, clinical chemistry, haematological variables, enzyme functions or organ weights. However, 3 weeks administration of HFC at 150 or 500 mg/kg bw daily (providing 71.8 or 239.3 mg/kg bw of humifulvate daily) was associated with decreases in body weights and in liver and kidney weights, which the authors attributed to undocumented reductions in appetite. Because no histopathology was performed, it could not be established whether the effects on kidney and

liver function were adverse or not. Thus the Panel derives a NOAEL, from this study, of 50 mg HFC/kg bw/day.

The petitioner also described results from a 6-month (180-day) repeated dose oral toxicity study of supplemented potassium humate powder in beagle dogs. The Panel derives a NOAEL for supplemented potassium humate powder from this study of 15 mg/kg bw/day equivalent to 7 mg HFC/kg bw/day.

The Panel concludes that the margin of safety between the NOAEL from the 28-day toxicity study in rats and the estimated human exposure amounts to only 8.8 and between the NOAEL from the 180-day beagle dog study and the estimated human exposure to 1.2. Therefore, the Panel concludes that the submitted data are insufficient to demonstrate the safety of the proposed use and use levels of iron(II)-, chromium(III)- and selenium humic acid/fulvic acid chelate and supplemented humifulvate.

CONCLUSIONS

The Panel concludes that the bioavailability of iron, chromium, selenium and other minerals from the humic acid/fulvic acid chelates (HFCs) and supplemented (dotated) humifulvate has not been demonstrated, and that the actual level of saturation of the humic acid/fulvic acid chelates (HFCs) and supplemented (dotated) humifulvate has also not been characterised.

The Panel also concludes the following:

- the bioavailability of iron, chromium, selenium or other minerals from their humic acid/fulvic acid chelates might be limited or even absent, whereas the possibility that the source may reduce the bioavailability of the metals and nutrients from other sources in the diet cannot be excluded.
- the specifications provided may only relate to the material described in the present Opinion originating from peat found primarily along the northern shores of Lake Balaton in Hungary;
- the Margin Of Safety between the NOAEL from the 28-day toxicity study in rats and the estimated human exposure amounts to only 8.8, and between the NOAEL from the 180-day beagle dog study and the estimated human exposure to 1.2.

Therefore, the Panel concludes that the submitted data are insufficient to demonstrate the safety of the proposed use and use levels of iron(II)- chromium(III)- and selenium humic acid/fulvic acid chelate and supplemented (dotated) humifulvate.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier on chromium(III)- humic/fulvic acid chelate. February 2005. Submitted by Humic 2000 Co Ltd.
2. Dossier on iron(II)- humic/fulvic acid chelate. February 2005. Submitted by Humic 2000 Co Ltd.
3. Dossier on selenium-humic/fulvic acid chelate. February 2005. Submitted by Humic 2000 Co Ltd.
4. Dossier on supplemented (dotated) humifultate. February 2005. Submitted by Humet Plc.

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GLOSSARY / ABBREVIATIONS

ANS	Panel on Food Additives and Nutrient Sources added to Foods
bw	Body weight
CAS	Chemical Abstracts Service
EC	European Commission
EFSA	European Food Safety Authority
EVM	Expert group on Vitamins and Minerals
FDA	U.S. Food and Drug Administration
FNB	Food and Nutrition Board
GLP	Good Laboratory Practice
HFC	Humic acid/Fulvic acid chelates
ICP-AES	Inductively Coupled Plasma - Atomic Emission Spectroscopy
MPCEs	Micronucleated polychromatic erythrocytes
NOAEL	No Observable Adverse Effect Level
PRI	Population Reference Intake
SCF	Scientific Committee on Food
TDS	Total Diet Study
UL	Tolerable Upper Intake Level
WHO	World Health Organisation