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Application for the Approval of Clinoptilolite

Regulation (EC) No.258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredients

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Introduction

Approval is sought under *Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredients*, for the marketing of Clinoptilolite, a naturally occurring zeolite aluminosilicate mineral, as a food supplement.

Regulation 258/97 applies "to the placing on the market within the Community of foods and food ingredients which have not hitherto been used for human consumption to a significant degree within the Community and which fall under the following categories … (c) with a new or intentionally modified primary molecular structure." Clinoptilolite is 'novel' as defined by virtue of its primary molecular structure.

Zeolites are a family of crystalline aluminosilicate minerals (Harben, 1999). The first zeolite was described in 1756 by Cronstedt, a Swedish mineralogist who coined the name from two Greek words meaning 'boiling stones', referring to the evolution of steam when the rock is heated. About fifty different natural zeolites are now known and more than one hundred and fifty have been synthesised for specific applications such as industrial catalysis or as detergent builders. Clinoptilolite is a naturally occurring zeolite, formed by the devitrification (ie the conversion of glassy material to crystalline material) of volcanic ash in lake and marine waters millions of years ago. It is the most researched of all zeolites and is widely regarded as the most useful.

In common with other zeolites, clinoptilolite has a cage-like structure consisting of SiO_4 and AlO_4 tetrahedra joined by shared oxygen atoms (Mumpton, 1983). The negative charges of the AlO_4 units are balanced by the presence of exchangeable cations - notably calcium, magnesium, sodium, potassium and iron. These ions can be readily displaced by other substances, for example heavy metals and ammonium ions (Semmens, 1983). This phenomenon is known as cation exchange, and it is the very high cation exchange capacity of clinoptilolite which provides many of its useful properties.

Clinoptilolite is currently used in diverse applications such as drinking water purification and as an animal feed additive (Mumpton, 1983 & 1999; Minato, 1976). Many studies have shown that clinoptilolite absorbs toxins created by molds in animal feeds, as well as enhancing nutrient absorption by cattle, pigs, lambs and other animals. Clinoptilolite of volcanic origin has been approved by the EU for use in the category of "Binders, anti-caking agents and coagulants" in feedingstuffs for pigs, rabbits and poultry at levels of up to 20,000 mg/kg (Commission Regulation, 2001).

Clinoptilolite forms the basis of the anti-diarrhoea drug 'Enterex', which was approved by the Cuban Drug Control Agency in 1995 (Rodriguez-Fuentes, 1997).

The large majority of toxicology studies on zeolites have been performed on clinoptilolite and Zeolite A – the latter because of its widespread use in household detergents. No fatal case arising from the oral uptake of either of these zeolites has been identified.

Under the *Commission recommendation of 29 July 1997*, section 4, the "Scientific Classification of Novel Foods for the Assessment of Wholesomeness", Clinoptilolite would be classified as Class 2.2, "Complex Novel Food from non-GM Source", "the source of the NF has no history of use in the Community." The requirements for this submission for this class are as follows:

I Specification of the Novel Food

II Effect of the Production Process Applied to the Novel Food

III History of the organism used as the source of the Novel Food
NOT APPLICABLE TO CLINOPTILOLITE

IV Effect of the Genetic Modification on the Properties of the Host Organism
NOT APPLICABLE TO CLINOPTILOLITE

V Genetic Stability of the GMO Used as Novel Food Source
NOT APPLICABLE TO CLINOPTILOLITE

VI Specificity of Expression of Novel Genetic Material
NOT APPLICABLE TO CLINOPTILOLITE

VII Transfer of Genetic Material From GMO
NOT APPLICABLE TO CLINOPTILOLITE

VIII Ability of the GMO to Survive In and Colonise the Human Gut
NOT APPLICABLE TO CLINOPTILOLITE

IX Anticipated Intake / Extent of Use of the Novel Food

X Information from previous human exposure to the Novel Food or its source
In the above mentioned SCF Guidelines this section is omitted, assuming that Clinoptilolite has not been consumed in the European Community. However, clinoptilolite has been consumed in other regions of the world, so it is relevant to include this section.

XI Nutritional Information on the Novel Food

XII Microbiological Information on the Novel Food

XIII Toxicological Information on the Novel Food

I SPECIFICATION OF THE NOVEL FOOD

Description of Clinoptilolite (Novel Food)

Clinoptilolite, which in Greek means 'oblique feather stone', is a member of the zeolite group of minerals (Harben, 1999). Zeolites are crystalline aluminosilicates whose framework consists of SiO_4 and AlO_4 tetrahedra joined by shared oxygen atoms (Mumpton, 1983). The negative charges of the AlO_4 units are balanced by the presence of exchangeable cations, primarily of group IA and IIA elements. Being a naturally occurring mineral, the precise composition of clinoptilolite is subject to a degree of variation. However, an approximate empirical formula is $(Ca, Fe, K, Mg, Na)_{3\text{-}6}Si_{30}Al_6O_{72}.24H_2O$. The Chemical Abstracts Service (CAS) Number for clinoptilolite is **12173-10-3**.

Euremica Environmental Ltd proposes to market clinoptilolite in capsules as a food supplement only. The proposed trade name for Clinoptilolite capsules is Zeolife®.

Source of Clinoptilolite

Deposits of clinoptilolite exist in many countries around the world, including the USA, Cuba, Italy, Greece, Ukraine and Japan. Euremica Environmental Ltd currently imports clinoptilolite only from a single mine at Duaringa, Queensland, Australia. This deposit is a very high purity clinoptilolite and, unlike many deposits, contains only very low levels of lead. In the event of alternative source(s) being utilised in the future, the mineral will of course be subjected to the same rigorous quality control procedures.

Quality Control Specifications and Methods

Micronized Clinoptilolite is a pinkish powder, its colour being conferred by its iron content. The rock is crushed, milled and packed in Australia prior to shipping. Since all the clinoptilolite is sourced from a single deposit, we anticipate that there will be little interbatch variation. However, every batch will be analysed – initially by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) at Durham University's Department of Earth Sciences, although other laboratories may be used in the future. Any batch found to contain an unacceptable level of any element will be rejected at this stage and not be used for human consumption.

The Novel Food is unlikely to contain any microorganisms of adverse public health significance. Nevertheless, each batch of clinoptilolite will undergo microbiological testing prior to the manufacture of capsules. Testing of two samples taken from the first batch of clinoptilolite has been completed by Q Laboratories, Quayside, Navigation Way, Ashton-on-Ribble, Preston, Lancashire PR2 2YP. Escherichia coliforms and Staphylococcus aureus were absent from both samples. Salmonella was not detected in either sample. Aerobic colony count and yeast were each <10 cfu/g in both samples. The mould counts were 100 cfu/g and <10 cfu/g for the two samples.

Incorporation of the clinoptilolite into capsules for human consumption as a food supplement will be carried out by Thompson & Capper Ltd or other UK-based Medicines and Healthcare Products Regulatory Agency (MHRA) licensed facility in accordance with Good Manufacturing Practice (GMP). The capsules will contain 250mg of clinoptilolite (particle size 30-50 microns), together with flow improvers (340mg rice flour, 6mg magnesium stearate, 6mg silicon dioxide). The capsule shell ingredients are gelatine and water. Since the non-clinoptilolite components of the capsules are standard ingredients, they have not been subjected to compositional analysis.

Analysis of Clinoptilolite by Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Five 0.1gram samples from a single batch of clinoptilolite were each dissolved with 1ml of 69% Aristar grade nitric acid and 4ml of Aristar grade hydrofluoric acid in Teflon vials. The process was also carried out on three reaction vials lacking clinoptilolite, to provide reagent blanks.

After dissolution, the solution is evaporated to dryness to remove volatile fluorides and residual hydrofluoric acid. Residual nitrate salts were dissolved in 5% Aristar grade nitric acid. These solutions were diluted 10-fold and analysed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Johnson, 2003).

Table 1. Analysis of Clinoptilolite by Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Number	Element	Concentration
1	H	Not Analysed For
2	He	Not Analysed For
3	Li	5.8ppm
4	Be	0.3ppm
5	B	1.0ppm
6	C	Not Analysed For
7	N	Not Analysed For
8	O	Not Analysed For
9	F	Not Analysed For
10	Ne	Not Analysed For
11	Na	0.39%
12	Mg	0.69%
13	Al	4.3%
14	Si	Not Analysed For
15	P	310ppm
16	S	Not Analysed For
17	Cl	Not Analysed For
18	Ar	Not Analysed For
19	K	1.1%
20	Ca	2.0%
21	Sc	7.4ppm

Number	Element	Concentration
22	Ti	0.21%
23	V	25ppm
24	Cr	8.3ppm
25	Mn	520ppm
26	Fe	1.4%
27	Co	3.2ppm
28	Ni	1.6ppm
29	Cu	14ppm
30	Zn	55ppm
31	Ga	25ppm
32	Ge	1.2ppm
33	As	4.0ppm
34	Se	Not Detected
35	Br	Not Analysed For
36	Kr	Not Analysed For
37	Rb	54ppm
38	Sr	0.11%
39	Y	32ppm
40	Zr	79ppm
41	Nb	7.6ppm
42	Mo	Not Detected
43	Tc	Not Detected
44	Ru	Not Detected
45	Rh	0.025ppm
46	Pd	0.88ppm
47	Ag	0.17ppm
48	Cd	0.18ppm
49	In	Not Analysed For
50	Sn	4.0ppm
51	Sb	0.71ppm
52	Te	0.005ppm
53	I	0.1ppm
54	Xe	Not Detected
55	Cs	3.6ppm
56	Ba	0.18%
57	La	38ppm
58	Ce	94ppm
59	Pr	14ppm
60	Nd	45ppm
61	Pm	Not Analysed For
62	Sm	8.4ppm
63	Eu	1.3ppm
64	Gd	8.1ppm
65	Tb	1.2ppm
66	Dy	7.3ppm
67	Ho	1.3ppm
68	Er	4.0ppm
69	Tm	0.64ppm

Number	Element	Concentration
70	Yb	4.8ppm
71	Lu	0.65ppm
72	Hf	3.9ppm
73	Ta	1.1ppm
74	W	0.6ppm
75	Re	Not Detected
76	Os	Not Detected
77	Ir	0.009ppm
78	Pt	0.03ppm
79	Au	Not Detected
80	Hg	0.028ppm
81	Tl	0.30ppm
82	Pb	31ppm
83	Bi	0.62ppm
84	Po	Not Analysed For
85	At	Not Analysed For
86	Rn	Not Analysed For
87	Fr	Not Analysed For
88	Ra	Not Analysed For
89	Ac	Not Analysed For
90	Th	48ppm
91	Pa	Not Analysed For
92	U	4ppm
93	Np	Not Analysed For
94	Pu	Not Analysed For
95	Am	Not Analysed For
96	Cm	Not Analysed For
97	Bk	Not Analysed For
98	Cf	Not Analysed For
99	Es	Not Analysed For
100	Fm	Not Analysed For
101	Md	Not Analysed For
102	No	Not Analysed For
103	Lr	Not Analysed For
104	Rf	Not Analysed For
105	Db	Not Analysed For
106	Sg	Not Analysed For
107	Bh	Not Analysed For
108	Hs	Not Analysed For
109	Mt	Not Analysed For

One gram of clinoptilolite contains 31µg lead, 0.18µg cadmium, 0.028µg mercury and 4µg of arsenic. The Provisional Tolerable Weekly Intake (PTWI) of these elements is 25, 7, 5 and 15µg/kg body weight respectively. The PTWI's are 6.9, 333, 1530 and 32 times greater, respectively, than the quantities consumed in 7grams (one week's recommended intake) as consumed by a 60kg individual, assuming 100% solubilisation of these metals in the stomach. In reality, only a small proportion of each of these elements is likely to be solubilised (see Section XIII).

Analysis of Clinoptilolite for Silicon and Sulphur by Scanning Electron Microscope With Energy Dispersive Analysis Through X-Ray Facility (SEM-EDAX)

The silicon and sulphur content of clinoptilolite has been determined at the University of Durham's Department of Earth Sciences by Scanning Electron Microscope With Energy Dispersive Analysis Through X-Ray Facility (SEM-EDAX) (Johnson, 2003). The Scanning Electron Microscope used was a Cam Scan Series 2, and the EDAX was operated using WinEDS (version 3.0) software. The operating conditions used were an accelerating voltage of 15kV and an acquisition live time of 20 minutes. X-ray spectra were acquired for 0.5 x 0.5mm areas and the results then fitted using existing data as internal standards. The following results were obtained:

Table 2. Analysis of Clinoptilolite for Silicon and Sulphur

Number	Element	Concentration
14	Si	26.9% w/w (+/- 1% w/w)
16	S	300ppm (+/- 50ppm)

Analysis of Clinoptilolite for F, Br and Cl by XRF

The F, Br and Cl content of clinoptilolite has been determined at the University of Durham's Department of Earth Sciences by XRF (Johnson, 2003). The sample was prepared by mechanically compressing the powder to form a pellet, which was then analysed using a Philips PW1400 Wavelength Dispersive X-Ray Fluorescence Spectrometer. The instrument was calibrated using fifteen certified reference materials and spiked standards prepared and analysed in an identical manner. The following results were obtained:

Table 3. Analysis of Clinoptilolite for F, Br and Cl

Number	Element	Concentration
9	F	< 200 ppm
17	Cl	< 5 ppm
35	Br	6 ppm

Analysis of Clinoptilolite for Dioxins by Micromass Ultima NT

The dioxin content of clinopilolite has been determined by Marchwood Scientific Services, Unit 4G Marchwood Industrial Park, Marchwood, Southampton SO40 4PB, using Micromass Ultima NT (Test Certificate No. 103-463). The analysis was done by high resolution gas chromatography following the US EPA1613 methodology for analysis of dioxins and furans in solid materials. The laboratory is UKAS accredited.

Table 4. Analysis of Clinoptilolite for Dioxins

The following concentrations are expressed as nanograms per gram:

Congener	Conc	TEFs	TEQ^1	TEQ^2	DL
2378-TCDF	*	0.100	0.0000	0.0000	0.0002
12378-PCDF	*	0.050	0.0000	0.0000	0.0002
23478-PCDF	*	0.500	0.0001	0.0000	0.0002
123478-HxCDF	*	0.100	0.0000	0.0000	0.0003
123678-HxCDF	0.0003	0.100	0.0000	0.0000	0.0002
234678-HxCDF	0.0005	0.100	0.0000	0.0000	0.0002
123789-HxCDF	*	0.100	0.0000	0.0000	0.0002
1234678-HpCDF	0.0007	0.010	0.0000	0.0000	0.0004
1234789-HpCDF	*	0.010	0.0000	0.0000	0.0004
OCDF	0.0008	0.001	0.0000	0.0000	0.0004
2378-TCDD	*	1.000	0.0001	0.0000	0.0001
12378-PCDD	*	0.500	0.0001	0.0000	0.0002
123478-HxCDD	*	0.100	0.0000	0.0000	0.0003
123678-HxCDD	*	0.100	0.0000	0.0000	0.0004
123789-HxCDD	*	0.100	0.0000	0.0000	0.0004
1234678-HpCDD	0.0013	0.010	0.0000	0.0000	0.0006
OCDD	0.0015	0.001	0.0000	0.0000	0.0012

Key To Table 4:

TCDF	Tetrachlorodibenzofuran
PCDF	Pentachlorodibenzofuran
HxCDF	Hexachlorodibenzofuran
HpCDF	Heptachlorodibenzofuran
OCDF	Octachlorodibenzofuran
TCDD	Tetrachlorodibenzo-p-dioxin
PCDD	Pentachlorodibenzo-p-dioxin
HxCDD	Hexachlorodibenzo-p-dioxin
HpCDD	Heptachlorodibenzo-p-dioxin
OCDD	Octachlorodibenzo-p-dioxin
*	Isomer Not Detected
TEQ	Toxic Equivalent Value
TEF	Toxic Equivalent Factor
Conc	Concentration
DL	Detection Value
TEQ^1	Concentration of Non Detected Congeners at Detection Limit
TEQ^2	Concentration of Non Detected Congeners at Zero

One gram of clinoptilolite contains quantities of dioxins which have a Toxic Equivalent Value of 300 femtograms of 2378-TCDD (the most toxic dioxin). The new Tolerable Daily Intake for 2378-TCDD of 1 to 4 picograms per kilogram body weight, determined by the WHO at Geneva in 1998 (WHO, 1998), is 200 to 800 times greater than the Toxic Equivalent Value of dioxin in one gram of clinoptilolite, as consumed by a 60 kg individual.

Analysis of Clinoptilolite for Protein Content

To provide evidence of the low allergenic potential of clinoptilolite, a protein assay has been performed by the Faculty of Health and Life Sciences, de Montfort University, The Gateway, Leicester LE1 9BH, using the standard Bradford method (Bradford, 1976). One 500 mg sample of clinoptilolite was suspended in 10 ml distilled water, and shaken at 100 rpm at ambient temperature (20°C). After centrifugation at 3G for 10 minutes, 100µl of the supernatant was mixed with 3 ml of standard Sigma Bradford Reagent and the optical density measured at 595nm. A reference protein curve was prepared using Bovine Serum Albumin.

Table 5. Analysis of Clinoptilolite for Protein

0.7mg Protein per g Clinoptilolite

Discussion of Protein Content

The protein content of 0.7mg per gram of clinoptilolite is higher than expected. As a precaution, except where appropriate analysis proves the clinoptilolite to be protein free, it will be heated to at least 100°C to denature any protein prior to incorporation into capsules for human consumption.

II EFFECT OF THE PRODUCTION PROCESS APPLIED TO THE NOVEL FOOD

Mining and Milling of Clinoptilolite

Euremica Environmental Ltd currently sources clinoptilolite from a single open cut mine at Duaringa, Queensland, Australia. Supersorb Environmental NL is the owner of the mine and the associated crushing and screening plant.

The first stage of the mining clinoptilolite is to strip away the overburden with a bulldozer. The overburden is pushed into a heap for use later in the rehabilitation of the mine site. The clinoptilolite is then pushed up into large windrows, again by bulldozer, for loading and relocation by truck to the crushing and screening plant. Here, the rock is placed into a hopper from which it is fed into a jaw crusher, where it is crushed down to 60mm. After this it is run over a screen. Material over 20mm in size goes back through the jaw crusher, and particles under 20mm go into a cone crusher and then to a hammermill. A ball mill is used to achieve a particle size of 30-50 microns. The clinoptilolite is then bagged and shipped to the UK.

By virtue of the unique nature of the product, which has no traditional counterpart, no history of a similar production process for any other food is known. However, no toxicological, nutritional or microbiological hazard is likely to arise from this process, nor has the process any known potential to alter the levels of substances with an adverse effect on public health.

Manufacture of Zeolifeâ Capsules

Zeolife® capsules will be manufactured by Thompson and Capper Ltd or other UK-based Medicines and Healthcare Products Regulatory Agency (MHRA) licensed facility in accordance with Good Manufacturing Practice (GMP).

As a precaution, except where appropriate analysis proves the clinoptilolite to be protein free, it will be heated to at least 100°C to denature any protein prior to incorporation into capsules.

Each capsule will contain:

340 mg Rice Flour
250 mg Clinoptilolite
6 mg Silicon Dioxide
6 mg Magnesium Stearate

The silicon dioxide and magnesium stearate are incorporated as “flow agents”. They are added to food supplement capsules to prevent the other ingredients clumping, thereby helping to produce capsules of uniform dosage. Rice flour is used as an inactive “filler”, again to ensure uniform dosage of the active ingredient.

The capsule shell is made of gelatine.

III History of the Organism used as the source of the Novel Food
NOT APPLICABLE TO CLINOPTILOLITE

IV Effect of the Genetic Modification on the Properties of the Host Organism
NOT APPLICABLE TO CLINOPTILOLITE

V Genetic Stability of the GMO Used as Novel Food Source
NOT APPLICABLE TO CLINOPTILOLITE

VI Specificity of Expression of Novel Genetic Material
NOT APPLICABLE TO CLINOPTILOLITE

VII Transfer of Genetic Material From GMO
NOT APPLICABLE TO CLINOPTILOLITE

VIII Ability of the GMM to Survive In and Colonise the Human Gut
NOT APPLICABLE TO CLINOPTILOLITE

IX ANTICIPATED INTAKE / EXTENT OF USE OF THE NOVEL FOOD

Background

It is well established that clinoptilolite strongly binds heavy metals such as lead, cadmium, mercury and nickel (Mondale et al, 1995 and Harben, 1999). These metals have no known biological role, and indeed are highly toxic. Further, many published studies have demonstrated that clinoptilolite binds a range of mycotoxins, benefiting the health of animals into whose feed clinoptilolite has been added (Huwig, 2001 and Phillips, 1990). There is also evidence that a dietary intake of clinoptilolite may strengthen the immune system (Pavelic et al, 2001).

Anticipated Intake of the Novel Food

Euremica Environmental Ltd is seeking approval for Clinoptilolite only as a food supplement. It is proposed to manufacture capsules each containing 250mg of micronised clinoptilolite. They will be sold in tubs containing 120 capsules. Each tub will bear directions to take two capsules in the morning and two in the evening, totalling four capsules (equivalent to 1 gram of clinoptilolite) per day.

Product Labelling

The product label will include the following wording:

Zeolifeâ , part of the Euremica Environmental range, is a natural supplement that contains micronised zeolite. Taken regularly as part of a balanced diet, it helps to maintain a healthy body.

INGREDIENTS Rice Flour, Zeolite, Capsule Shell (Gelatine, Water), Magnesium Stearate, Silicon Dioxide.

NUTRITIONAL INFORMATION

Ingredient	Typical Weight	%RDA
Zeolite	250mg	N/A

DIRECTIONS FOR USE Swallow four capsules per day with liquid, two in the morning and two in the evening. This container provides 30 days' supply.
DO NOT EXCEED THE RECOMMENDED DAILY INTAKE

WARNING If you are pregnant, nursing, taking medication or have a medical condition, consult your doctor before taking this product. Discontinue use if you notice any unusual effects.
KEEP OUT OF REACH OF CHILDREN.

Anticipated Extent of Use of the Novel Food

It is the Company's intention to promote Zeolife® capsules by a series of advertorial features in health magazines, womens' magazines and other publications. The product will also be promoted on the Company's website, which will offer customers the facility to order on-line. We believe that Zeolife® capsules will come to be recognised as offering genuine benefits for most adults as part of a balanced diet, helping to maintain a healthy body by adsorbing and accelerating the elimination of heavy metals and mycotoxins, as well as by providing a source of silicon. It is anticipated that many supermarket, pharmacy and other shop chains will wish to stock the capsules and that it will become a popular product amongst the supplement-buying public.

X INFORMATION FROM PREVIOUS HUMAN EXPOSURE TO THE NOVEL FOOD OR ITS SOURCE

Studies reporting significant previous human exposure to clinoptilolite within the EU have not been identified. However, in Bulgaria, pills and biscuits were prepared for human consumption to adsorb heavy metal radioisotopes consumed with contaminated food after the Chernobyl disaster (Mumpton, 1999). More recently, the clinoptilolite-based anti-diarrheic drug "Enterex" was approved by the Cuban Drug Control Agency in 1995 (Rodriguez-Fuentes et al, 1997).

Four clinical studies have been reported for Enterex (Rodriguez-Fuentes et al, 1997), and details of these are included below to demonstrate that hundreds of patients have undergone clinical trials with no adverse effects being observed.

In the first study, sixteen men and fourteen women were each treated with between 2 and 6 Enterex tablets at the rate of 2 tablets every 4 hours. (Each tablet contained 900 mg clinoptilolite.) This study established that 4 to 6 tablets comprises an effective dose.

In a second study, 73 volunteer patients with acute diarrhoea were treated with Enterex using the doses established in the first study. These patients were also subjected to studies to establish the cause of the diarrhoea. The pathogens (Shigella, Entoamoeba histolytica and Giardia lamblia) were treated with antibiotics after the treatment with Enterex. The presence of clinoptilolite in the digestive tract did not disrupt the efficacy of the antibiotic treatment, an advantage over kaolin-based drugs.

The third study was a comparative study in which diabetic patients with vascular impairments (neuropathic diarrhoea) were given either Enterex or diphenoxilate of atropine, an antimotility drug. Due to the vascular impairments, recovery of patients must be achieved within 24 hours. The results revealed no significant difference between the two drugs. Further, the study demonstrated that a second dose of Enterex had no adverse side effects, a benefit which diphenoxilate of atropine could not boast.

In a fourth study, 434 volunteers with acute diarrhoea resulting from food intoxication (the main cause of acute diarrhoea in adults) were treated with Enterex. Three quarters (75.6%) of the patients recovered from the diarrhoea within 24 hours, and the remaining 24.4% recovered during the following 12 hours. These figures confirm the results obtained in the earlier studies.

Most patients showed good tolerance to treatment with Enterex, and none dropped out of the four clinical trials due to side effects.

Drug Interactions

There is no interaction between Enterex and Tetracycline or Chloramphenicol, the antibiotics normally used in the treatment of patients affected by enterobacteria such

as *Vibrio cholerae* serogroup 01 (Rodriguez-Fuentes et al, 1997). Also, it has been reported that clinoptilolite can be administered simultaneously with metronidazole and sulfamethoxazole without any loss of individual pharmaceutical effects (Farias et al, 2002). In vitro tests have revealed a low level of adsorption of aspirin, thyophiline, propanolol and phenobarbital on clinoptilolite (Rodriguez-Fuentes et al, 1997).

The proposed product label will include the following wording:

WARNING If you are pregnant, nursing, taking medication or have a medical condition, consult your doctor before taking this product. Discontinue use if you notice any unusual effects.

XI NUTRITIONAL INFORMATION ON THE NOVEL FOOD

Euremica Environmental Ltd proposes to sell Clinoptilolite in capsule form as a food supplement. It is not intended to replace any food in the diet.

Heavy Metal Adsorption

The fundamental building blocks of which zeolites are composed are tetrahedra of AlO_4 and SiO_4 with the Al or Si at the centre of each tetrahedron (Mumpton, 1983). The SiO_4 units are electrically neutral, but each AlO_4 unit carries a negative charge, creating fixed charged sites throughout the crystal structure. To maintain electroneutrality, the negative charges are associated with an equivalent number of cations - notably calcium, magnesium, sodium, potassium and iron in the case of clinoptilolite from the Duaringa mine in Australia. In a process known as cation exchange, these inocuous cations can be readily displaced by other substances, especially the heavy metals lead, cadmium, mercury and nickel for which clinoptilolite has a high affinity. The health problems associated with lead (from sources such as old water pipes), cadmium (for example from car exhaust catalysts) and other heavy metals are well known. Due to the unique pore-and-channel structure of clinoptilolite, cation exchange can occur throughout the entire crystal structure, leading to the adsorption and removal of heavy metals from the body.

Cadmium

The oral intake of cadmium from food and water is estimated to be 10-35 μg per day (WHO, 1993). Smoking provides a significant additional source of this metal. Cadmium has a long biological half-life in humans of 10 – 35 years and accumulates primarily in the kidneys, which become the main target organs for cadmium toxicity. Assuming an absorption rate for dietary cadmium of 5% and a daily excretion rate of 0.005% of body burden, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that, if levels of cadmium in the renal cortex are not to exceed 50 mg/kg, the total intake of cadmium should not exceed 1μg/kg body weight per day. The provisional tolerable weekly intake (PTWI) was therefore set at 7μg/kg body weight. It is recognised that the margin between the PTWI and the actual weekly intake of cadmium by the general population is small, less than 10-fold, and that this margin may be even smaller in smokers.

Additionally, recent research has shown that cadmium could also cause cancer (Jin et al, 2003). Cadmium not only damages DNA directly, but it has also been found to inhibit DNA repair, increasing mutation rates by as much as 2,000 fold. The amount of cadmium required to inhibit repair is very small, and may well be relevant to cadmium industry workers and smokers.

The protection afforded by clinoptilolite has been demonstrated in weanling Landrace x Yorkshire pigs (Pond and Yen, 1983). The pigs were fed a basal diet containing 3% clinoptilolite or zeolite A with or without 150ppm cadmium chloride for 31 days. Pigs fed cadmium in the absence of a zeolite had depressed levels of

haematocrit and haemoglobin; pigs fed cadmium in the presence of either zeolite did not. It was found that liver cadmium concentrations were increased dramatically by the addition of cadmium to the diet but this effect was significantly reduced in animals also fed with clinoptilolite.

Lead

Lead is present in tap water to some extent as a result of dissolution from natural sources, but primarily from household plumbing systems containing lead in pipes, solder, fittings, or the service connections to homes (WHO, 1993). The amount of lead dissolved from the plumbing system depends on several factors, including pH, temperature, water hardness, and standing time of the water, with soft, acidic water being the most plumbosolvent.

Lead is toxic to both the central and peripheral nervous systems. It also interferes with calcium metabolism, both directly and by interfering with vitamin D metabolism.

Renal tumours have been induced in experimental animals exposed to high concentrations of lead compounds in the diet, and IARC has classified lead and inorganic lead compounds in Group 2B (possible human carcinogen). However, there is evidence from studies in humans that adverse neurotoxic effects other than cancer may occur at very low lead concentrations (WHO, 1993).

Concentrations of lead in the livers and kidneys of pigs have been significantly reduced by the addition of 1.0% clinoptilolite to diets containing 500 or 1000ppm lead (Pond et al, 1996).

It is believed that the high affinity of clinoptilolite for lead would significantly reduce the amount of dietary lead available for absorption by the body.

Mycotoxin Chemisorption

Clinoptilolite is known to bind a range of mycotoxins, forming highly stable complexes (Tomasevic-Canovic et al, 2001 and Huwig et al, 2001). Mycotoxins are a diverse family of toxins produced by certain fungi, especially by species of Aspergillus, Fusarium, Penicillium, Claviceps and Alternaria. There are several hundred distinct mycotoxins, capable of causing health problems such as renal and hepatic syndromes and diminished immunocompetency. The most extensively researched mycotoxins are the aflatoxins. The aflatoxins are characterised by the presence of a di- or tetrahydrofurofuran containing a substituted coumarin and a terminal cyclopentenone (B-type aflatoxins) or a lactone (G-type aflatoxins). They are potent carcinogens. The ability of clinoptilolite to adsorb the aflatoxins has resulted in measurable improvements in the health of swine, sheep, chickens and quail (Mumpton, 1999; Parlat et al, 1999; Kyriakis et al, 2002).

In one study (Parlat et al, 1999), clinoptilolite was incorporated into the diets of Japanese quail chicks (*Coturnix coturnix japonica*) at the rate of 50g/kg and evaluated for its ability to reduce the deleterious effects of 2.0mg total aflatoxin per

kilogram of feed (a mixture of aflatoxins B_1, B_2, G_1 and G_2 was used). A total of forty birds were divided into four treatment groups (control, aflatoxin, clinoptilolite, aflatoxin plus clinoptilolite diets). The aflatoxin treatment significantly decreased food consumption and weight gain from the third week onwards. The adverse effect of aflatoxin on food conversion ratio was also significant from the fourth week of the experiment. However, addition of clinoptilolite to an aflatoxin-containing diet significantly reduced the deleterious effects of the toxin on food consumption, weight gain and food conversion ratio. Food consumption was reduced by 14% in quail chicks consuming the aflatoxin diet, but by only 6% for quail chicks consuming the aflatoxin plus clinoptilolite diet. Similarly, weight gain was reduced by 27% in birds consuming the aflatoxin diet, but by only 8% for birds consuming the aflatoxin plus

clinoptilolite diet. No mortality was observed in any of the groups.

In a further study, the effect of a clinoptilolite-enriched diet on the toxic effects of ingested mycotoxins (zearalenone and trichothecene) in swine was investigated (Kyriakis et al, 2002). Two hundred and forty crossbred gilts and sows (Large White x Landrace) were divided equally into two groups. Clinoptilolite was incorporated into the diet of one group at the rate of 2%. The clinoptilolite-enriched diet was shown to reduce the prevalence of anoestrous (7.3% compared with 15.8%), the proportion of litters with splay-legged piglets (9.9% compared with 17.5%), and the proportion of litters with female piglets showing vulvovaginitis (4.5% compared with 14.5%).

Micronutrients and Vitamins

It has been demonstrated that clinoptilolite binds the essential micronutrients copper, zinc, cobalt and manganese to only a very limited extent (Tomasevic-Canovic et al, 2001). Chemisorption indexes were obtained using a solution chosen to simulate the gastric juice of animals. It contained 0.1mol per litre hydrochloric acid and 0.05mol per litre sodium chloride at pH 3.8 and a temperature of 37°C. A certain amount of each micronutrient (2 mg copper, 20mg zinc, 16mg manganese and 0.16 mg cobalt) was added to 0.1 litre of the electrolyte solution and an aliquot taken for the determination of the starting concentrations of the cations present in the solution. One gram of clinoptilolite was then added to the solution. After gentle shaking for two hours, the concentrations of the non-exchanged micronutrients were determined in the supernatant. Both the total and non-exchanged concentrations of the metals were determined by atomic absorption spectrophotometry.

The chemisorption indexes of each micronutrient were calculated according to the formula:

$$c_\alpha = (c_t - c_f) / c_t$$

where:
c_α = Chemisorption Index of Micronutrient
c_t = Total Concentration of Micronutrient
c_f = Concentration of Non-Adsorbed of Micronutrient.

A Chemisorption Index of 1 represents total adsorption (high affinity).
A Chemisorption Index of 0 represents no adsorption (zero affinity).

The Chemisorption Indexes obtained were as follows:

Cu – 0.23
Zn – 0.00
Co – 0.10
Mn – 0.03.

In a different study (Tomasevic-Canovic et al, 1996), one gram of clinoptilolite was added to 100 ml of synthetic gastric juice containing 16.5 mg/100ml phenylalanine, 20.6 mg/100ml tryptophan, 1.5 mg/100ml Vitamin A, 10 mg/100ml Vitamin E and 12.5 mg/100ml Vitamin D. The suspension was placed in a water bath at 37ºC and gently shaken for two hours. After this time, the clinoptilolite was separated by centrifugation, and the concentrations of non-adsorbed aminoacids and vitamins in the supernatant determined by HPLC. The data obtained show that clinoptilolite does not adsorb either of the amino acids nor any of the fat-soluble vitamins studied.

In another study (Kyriakis et al, 2002) the effect of the long-term use of clinoptilolite on the serum concentrations of certain nutrients (vitamins A and E, and inorganic phosphorus, potassium, copper and zinc) in sows was investigated. Twenty-four cross-bred sows (Large White x Landrace) participated in the study, divided equally into two experimental groups. Clinoptilolite was incorporated in the basal feed offered to one group at the inclusion rate of 2%. The feeds of both groups were supplemented with commercial vitamin and mineral premixes at the recommended inclusion rates. No significant alterations in the serum concentrations of any of the vitamins and minerals studied was observed.

Aluminium Bioavailability

Aluminium silicates, sulphates and phosphates are common food additives to which humans are exposed (Cefali et al, 1995). Aluminium is also present in a variety of natural food sources and in water supplies. Because of the limitations of the animal data and uncertainty surrounding human data, the WHO has not yet derived a health-based guideline value for aluminium. However aluminium and its compounds appear to be poorly absorbed in humans (WHO, 1998). Euremica Environmental's clinoptilolite contains 4.3% aluminium (see Table 1, page 7), of which 17.09% has been shown to be solubilised in in vitro gastric fluid extraction (see Table 7, page 25). This equates to a daily dose of 7.25mg of solubilised aluminium (based on our proposed intake instructions of four capsules each day), of which only a small proportion would be expected to be absorbed into the blood.

XII MICROBIOLOGICAL INFORMATION ON THE NOVEL FOOD

Being an aluminosilicate mineral of volcanic origin, the extent of contamination of the raw clinoptilolite by microorganisms and their metabolites at the outset of the production process would be expected to be low. Moreover, the clinoptilolite has an extremely low water content, effectively inhibiting the growth of typical food-borne microbes. Nevertheless, each batch will be subjected to microbiological testing to ensure safety.

Testing of two samples taken from the first batch of clinoptilolite has been completed by Q Laboratories, Quayside, Navigation Way, Ashton-on-Ribble, Preston, Lancashire PR2 2YP. Escherichia coliforms and Staphylococcus aureus were absent from both samples. Salmonella was not detected in either sample. Aerobic colony count and yeast were each <10 cfu/g in both samples. The mould counts were 100 cfu/g and <10 cfu/g for the two samples.

The capsules will be manufactured by Thompson and Capper Ltd or other UK-based Medicines and Healthcare Products Regulatory Agency (MHRA) licensed facility, in accordance with Good Manufacturing Practice (GMP). The combination of the microbiological stability of clinoptilolite together with GMP standards of manufacture will ensure the microbiological safety of the product.

XIII TOXICOLOGICAL INFORMATION ON THE NOVEL FOOD

There is no traditional counterpart of the Novel Food that can be used as a baseline to facilitate the toxicological assessment, but information is available from a range of toxicological studies appropriate to the Novel Food to show that the Novel Food is safe under anticipated conditions of preparation and use.

Human clinical trials have been performed on Enterex (clinoptilolite) tablets. No adverse toxicological effects were reported (see section X, page 17).

No allergenic potential has been observed in any of the toxicology trials undertaken (Rodriguez-Fuentes et al, 1997 and Pavelic et al, 2001).

It should be noted that Commission Regulation (EC) No 2200/2001 of 17 October 2001 concerning provisional authorisations of additives in feedingstuffs permits the addition of clinoptilolite of volcanic origin to all feedingstuffs for pigs, rabbits and poultry up to a maximum content of 20,000 mg/kg.

Exchangeable Cations

A series of experiments has been undertaken at Durham University's Department of Earth Sciences to determine, for a range of metal cations, the maximum proportion that could be considered exchangeable (Johnson, 2003). The significance of this work is that it gives an indication of the theoretical maximum concentration of the metal ions which could be displaced from the clinoptilolite in the digestive tract. The principle is to contact the clinoptilolite with a saturating concentration of ammonium ions, for which clinoptilolite has a very high affinity, and which can be expected to displace all other exchangeable cations. (Because there is not a high concentration of ammonium ions in the human stomach, the amount of each cation to be exchanged from ingested clinoptilolite is much smaller – see section on Gastric Fluid Extractable Elements below.)

A 2 gram sample of clinoptilolite was thoroughly mixed with 20ml of 1molar Analar grade ammonium acetate. The mixture was centrifuged and the supernatant decanted. The supernatant was then diluted by a factor of 2000 to ensure a total concentration of dissolved solids of less than 0.1 gram per litre prior to analysis by Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

Table 6. Exchangeable Metals

Element	Whole Rock	Exchangeable Proportion (%)
Na	0.39%	50
Mg	0.69%	10
Al	4.3%	0
Ca	2.0%	43
Ti	0.21%	0.7
Mn	520ppm	0.5

Element	Whole Rock	Exchangeable Proportion (%)
Sr	0.11%	35
Y	32ppm	2.3
Ba	0.18%	24
La	38ppm	1.8
Pr	14ppm	3.8
Nd	45ppm	4.3

The amount of Zn, Cd, Pb, Ni and Cu in the supernatant were in each case below the detection limits. The quantity of these ions that could be expected to be displaced from clinoptilolite in the digestive tract would therefore be miniscule.

Gastric Fluid Extractable Elements

To investigate the amounts of various key elements that are potentially extractable from clinoptilolite in the stomach, a further series of experiments was conducted at Durham University's Department of Geological Sciences (Johnson, 2003).

One gram of clinoptilolite was mixed with 50ml of synthetic gastric fluid and placed in an oven at 38°C for two hours. The samples were shaken by hand every 20 minutes. The solutions were then filtered (using 0.45μm pore filters) and analysed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) or, in the case of the higher concentration elements, Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). The average results for 5 replicates, together with Standard Deviations, are presented below.

Samples are corrected for the metals content of the blank solutions which contained significant quantities of K, Cr and Cu – these are believed to have been present initially in the pepsin. This is not believed to have significantly affected the results presented for these elements.

The synthetic gastric fluid contained: 2.0g/l NaCl, 3.2g/l pepsin and 7.0ml/l HCl, - (US Pharmacopeia (1995) p2053). Where possible Analar grade reagents were used.

The synthetic gastric fluid was initially pH 1.2, compared with a final figure of pH 1.4.

Table 7. Gastric Fluid Extractable Elements

Element	Concentration in Gastric Fluid Solution	Standard Deviation	Percentage of Total in Zeolite*
Sb	Not detected	-	-
Hg	Not detected	-	-
Cd	0.5ppb	0.31ppb	13.9
Cr	4.1ppb	0.9ppb	2.5
As	12.5ppb	0.28ppb	15.6
Cu	19.5ppb	6.1ppb	7.0

Element	Concentration in Gastric Fluid Solution	Standard Deviation	Percentage of Total in Zeolite*
Ni	20.6ppb	1.0ppb	64.4
Co	21.8ppb	0.6ppb	34.1
Ti	62.5ppb	1.7ppb	0.1
Pb	91.8ppb	8.1ppb	14.8
Zn	164.7ppb	5.8ppb	15.0
P	4.4ppm	0.2ppm	70.0
Si	20.2ppm	0.6ppm	0.375
Mn	4.6ppm	0.1ppm	44.23
Ba	3.7ppm	0.4ppm	10.27
Sr	5.6ppm	0.2ppm	25.45
Fe	7.4ppm	0.3ppm	2.64
K	6.8ppm	1ppm	3.09
Mg	30.5ppm	1ppm	22.10
Al	147ppm	5ppm	17.09
Ca	158ppm	4ppm	39.5

* the percentage of each element found in solution relative to the total available from 1 gram based upon previous analysis.

It is important to note that this experiment used only 50 ml of the synthetic gastric fluid. In life, there would normally be a much greater dilution effect in the stomach due to the significantly higher volumes of gastric fluid naturally present and other liquids (consumers are directed to take the capsules with liquid). Additionally, the experiment used an amount of clinoptilolite equivalent to one daily dose (one gram of clinoptilolite, equivalent to four capsules), whereas consumers are directed to take half the dose in the morning and half the dose in the evening (See Section IX). There are therefore no safety concerns on the basis of the above data.

Animal Nutrition Applications

Experiments have been carried out in Japan since 1965 on the use of clinoptilolite as a dietary supplement for several types of animals. Over the years, much of this work has been repeated and enlarged upon by researchers in the USA, Australia and elsewhere, and serves to demonstrate the lack of toxic (and, indeed, the presence of beneficial) effects of clinoptilolite (Mumpton, 1985; Mumpton 1999 and references therein).

Studies have shown that the addition of clinoptilolite to the diets of swine, poultry, ruminants and other animals results in improved weight gain and feed conversion ratios (Kyriakis et al, 2002, and Mumpton, 1985). Milk yields are improved in dairy herds. In addition, the incidence of scours, enteritis and other intestinal diseases are decreased substantially (Mumpton, 1985).

Using 0%, 3%, 5% and 10% clinoptilolite as a feed supplement for Leghorn chickens, one study found that at all levels of inclusion, increased feed efficiency ratios were obtained compared with the control diet. Feedstuffs containing 10%

zeolite gave rise to efficiencies more than 20% greater than those of normal rations. (reviewed by Mumpton, 1985). No adverse effects on the health or vitality of the birds were observed.

In a further study, it was found that broiler chickens fed on a diet of 5% clinoptilolite gained slightly less weight over a two-month period than birds receiving a normal diet, but again average feed conversion ratios were higher (reviewed by Mumpton, 1985). None of the 48 test birds died during the experiment, while three on the control diet and two on the control diet supplemented with antibiotics succumbed.

There is evidence that addition of clinoptilolite to the rations of pregnant sows contributes to increased litter weights and healthier offspring (Mumpton, 1985). Experiments at the Ichikawa Livestock Experiment Station, where 400 grams of clinoptilolite was fed each day to pregnant sows and continued through the 35 day weaning period of their offspring, showed a substantial increase in the growth rate of the young pigs. Test animals weighed from 65 to 85 percent more than control group animals at the end of the weaning period. Young pigs whose dams received the clinoptilolite diet also suffered almost no attacks of diarrhoea, while those in control groups were severely afflicted with scours, greatly inhibiting their normal growth. The addition of 5% clinoptilolite to the rations of pregnant sows 20 to 90 days after mating gave rise to improved feed efficiency values (FEV = weight gain / feed intake) and increased litter weights at parturition. The earlier the clinoptilolite was added, the greater was the apparent effect.

A study (reviewed by Mumpton, 1985) evaluated the use of zeolites in the diets of young and mature Yorkshire pigs in 60 day and 79 day experiments, respectively, and found that the weight gain of animals of both ages receiving diets containing 5% clinoptilolite was 25 to 29% greater than that of animals receiving normal diets. Feed supplemented with zeolites gave rise to feed efficiencies about 35% greater than those of normal pigs, and about 6% greater when given to older animals. A further study found that the digestibility of crude protein and nitrogen-free extracts tended to be improved as zeolite was substituted for wheat bran in swine diets at levels of 1 to 6% over a 12 week period (Mumpton, 1985). Similar results were reported in a trial using 5% zeolite for 8 pigs over a 12 week period (Mumpton, 1985). Toxic or other adverse effects were not observed for any of these test animals. On the contrary, the presence of zeolite in swine rations appears to contribute measurably to the well-being of the animals. Tests carried out on 4000 head of swine in Japan showed a remarkable reduction in the incidence of disease and mortality among animals fed a diet containing 6% clinoptilolite compared with control animals over a twelve month period. There were only 22 cases of gastric ulcers (compared with 77 in the control trial), 51 cases of pneumonia (128 in the control trial), 4 cases of heart dilatation (6 in the control trial). The mortality rate was only 2.6, compared with 4.0 in the control trial (Mumpton, 1985).

Clinoptilolite has also proven to be effective in protecting animals against mycotoxins (Huwig et al, 2001 and Tomasevic-Canovic et al, 2001). The ability of clinoptilolite to absorb aflatoxins that contaminate animal feeds has resulted in measurable improvements in the health of swine, sheep and chickens (Mumpton, 1999).

Toxicology Studies

Clinoptilolite has been used as a vaccine adjuvant, glucose absorbent (potential medication for patients with diabetes mellitus) (Concepcion-Rosabal et al, 1997) and for the treatment of diarrhoea (see Section X) (Rodriguez-Fuentes et al, 1997). For this last application, the drug 'Enterex' was approved by the Cuban Drug Control Agency in 1995.

The chemical composition of the clinoptilolite used in "Enterex" has been reported as follows (Rodriguez-Fuentes et al, 1997):

Table 8. Composition of Clinoptilolite Used In Enterex

Element	Concentration
SiO_2	66.00%
Al_2O_3	10.96%
Fe_2O_3	1.80%
FeO	0.5%
MgO	0.90%
CaO	4.51%
Na_2O	0.97%
K_2O	1.00%
P_2O_5	0.05%
H_2O	4.83%
F	1ppm
Pb	2ppm
As	0.1ppm
Cd	0.5ppm
Hg	0.2ppm

The only active ingredient in Enterex is a purified natural clinoptilolite (Rodriguez-Fuentes, personal communication, 2003). The dosage of Enterex is two tablets (each containing 900mg of clinoptilolite) every four hours. Biological or toxic effects were not observed in the toxicological and preclinical tests. Adverse effects have not been observed in any of the six clinical studies conducted in patients with diarrhoea (Rodriguez-Fuentes, personal communication, 2003).

Acute, Subchronic and Chronic Oral Toxicity

Acute, subchronic and chronic oral toxicity was studied in mice and rats (Pavelic et al, 2001).

The mice were of the CBA/HZgr strain. Clinoptilolite was given mixed with standard food in the ratio of 25:75. The animals were monitored over the following periods: acute toxicity, 1 month; subchronic toxicity, 3 months; chronic toxicity, 6 months. Animals were monitored for: phenotypic changes, changes in behaviour, and survival (every day), changes in body weight (weekly), amount of food and water consumed (checked on days 14 and 28 when mice were kept for 24 hours in metabolic cages,

five mice per cage), changes in haematological and serum clinical chemistry parameters (erythrocytes, leukocytes, platelets, haematocrit, haemoglobin, glucose, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, bilirubin, inorganic phosphorus, and calcium; after 1,3 and 6 months); and urine clinical chemistry parameters (glucose, proteins, urobilinogen, bilirubin, nitrites, erythrocytes, leukocytes, pH and specific gravity; urine was collected while the animals were kept, once a month for 24 hours in metabolic cages). Pathohistological analysis of liver, spleen, kidney, brain, lung, testes, ovary, duodenum, eye, stomach, large and small intestine, muscles, myocardium, pancreas, thymus, axillary lymph node was carried out on killed experimental and control mice. No statistically significant changes were observed.

For the rat studies, Wistar rats were used. Clinoptilolite was given mixed with standard food at ratios of 25:75 and 50:50. The duration of the study was the same as for the mouse study. Animals were monitored for: phenotypic changes, changes in behaviour and survival (every day), changes in body weight (every 4 days), amount of food (every day) and water consumed (every 4 days), and changes in haematological and serum clinical chemistry parameters (the same as for mice; once a month). Pathohistological analysis of liver, spleen, lung, kidney, testes ovary and brain, was performed on killed experimental and control rats after 1, 6, and 12 months. No statistically significant changes were observed.

Carcinogenicity

Long term carcinogenicity trials have been carried out in Wistar rats (Tatrai and Ungvary, 1993). Respirable clinoptilolite (particle size below 5 μm) was administered intratracheally in doses of 0, 30 or 60 mg/animal to groups of 50 female and 50 male (except male controls which consisted of groups of 60) 5-week-old animals. Control animals were treated intratracheally with physiological saline. Treatments were performed in ether-anaesthetised animals, which were under constant observation, with clinical symptoms and death recorded daily. Body weights were measured once a week. Autopsies were performed immediately on rats that died or were killed during the study. Survivors were killed at the end of the study (104 weeks). The animals were studied both macroscopically and microscopically for the presence of neoplastic and non-neoplastic lesions. All organs were weighed and studied histologically. None of the experimental groups showed a significant increase in the incidence of any specific tumours compared to the corresponding control value, and no positive trend was noted in the occurrence of tumours (Cochran-Armitage linear trend test). The anatomical sites and histological characteristics of tumours were similar to those of spontaneous tumours occurring in Wistar rats. The results indicate that clinoptilolite has no carcinogenic activity in Wistar rats when administered intratracheally.

Reproductive and Developmental Toxicity

Reproductive and developmental toxicity trials have been performed on Sprague-Dawley rats (Pond and Yen, 1983b), CBA/HZgr mice (Pavelic et al, 2001b) and sows (Kyriakis et al, 2002).

The Sprague-Dawley rat study (Pond and Yen, 1983b) is the earliest paper identified to examine the effects of long-term ingestion of clinoptilolite on the growth and reproduction of animals. In this extensive study, female rats were grown from weaning using either a basal diet, or with 5% by weight clinoptilolite added. At about 13 weeks, a young male was placed with each female until mating. The pregnant females were continued on the same diets to which they had been assigned during growth. Several parameters were measured, including body weights after parturition and at 1, 2 and 3 weeks postpartum, number of pups born per litter, live birth weight and average total litter birth weight. Number of live pups and total litter weight were recorded at 1, 2 and 3 weeks postnatally. At three weeks, pups were weaned and the dams sacrificed using diethyl ether. Blood samples were obtained from the dams for determination of haemoglobin, haematocrit and plasma urea nitrogen. Livers and kidneys were weighed fresh and after drying. Two male and two female pups from each litter were randomly selected at weaning (3 weeks) and caged two per cage. Individual body weights were recorded weekly for 4 weeks, after which males were sacrificed, blood collected for haematocrit and liver, kidneys and testes removed and weighed. Young adult fertile males were introduced into each remaining cage until mating had occurred. Pregnant females were continued on their respective diets during gestation and were weighed immediately after parturition and one day postpartum. Numbers of total and live pups per litter were recorded, and total weight of each litter at birth and at one day of age was determined. Dams were sacrificed in the same manner as the first generation, blood haemoglobin and haematocrit were determined and liver and kidneys weighed. The data obtained from these trials demonstrates that long-term ingestion of clinoptilolite has no apparent deleterious effect on growth or reproduction of animals. There was no evidence of toxicity nor of teratogenicity of clinoptilolite added to the diet at 5% continuously during the growing period of female rats and throughout pregnancy and lactation. Furthermore, their offspring grew normally and had normal reproduction. It was concluded that clinoptilolite at 5% in the diet of the rat throughout the post-weaning portion of the life cycle and through one reproduction and lactation period was not associated with observable toxicological or teratogenic effects.

In the mouse studies (Pavelic et al, 2001), clinoptilolite was given mixed with standard food in a ratio of 25:75. For the reproductive toxicity study, ten male and ten female mice were fed with the food supplemented with clinoptilolite for 50 days and at least 14 days, respectively, before mating. The treatment continued during the prepregnancy and pregnancy period (one cycle) and until weaning. The same pair of animals was fed with clinoptilolite and monitored during four consecutive cycles (approx 4-5 months). The same schedule was applied for control animals. The parental generation was monitored for duration of cycle period (prepregnancy and pregnancy period), fertility (presence or absence of litter in a particular cycle), delivery incidence, mortality, and pathohistological appearance of ovaries, after the 4^{th} cycle. Number of total and viable pups born as well as gain in pups' body weight and pups' mortality until weaning was also scored. The prepregnancy period was shorter in the clinoptilolite-treated mice. The number of pups per litter was increased in clinoptilolite-treated mice, and probably for this reason the gain in body weight until weaning was decreased and a higher rate of mortality between days 8 and 21 of the neonatal period was observed. However, there were no differences between

control and treated animals that would suggest reproductive toxicity attributable to the clinoptilolite administration.

To study developmental toxicity, healthy pregnant mice were fed with clinoptilolite mixed with conventional food from day 6 to 16 of gestation and the mice were killed one day before parturition. The foetuses were analysed for microscopic pathology. No teratogenic effects were found.

In a Greek study (Kyriakis et al, 2002), 240 crossbred gilts and sows were divided equally into two groups, and 2% clinoptilolite incorporated into the feed of one group. The sows and gilts of each group were offered the experimental diets from weaning (or from age of six months for the gilts), during service, pregnancy and lactation and up to the date of service of the next reproductive cycle. All sows and gilts and their litters were monitored daily. No adverse or side effects were noted in the sows that were on the clinoptilolite-enriched diet. They showed normal oestrus behaviour during the breeding period, and gave rise to a slightly better farrowing rate (92.5%) compared to the control group (85.8%). No teratogenic effects were reported.

In a further study (Kyriakis et al, 2002), twenty-four crossbred sows (Large White x Landrace) were divided into two equal groups, the experimental group being offered feed incorporating 2% clinoptilolite. The study was maintained for a complete reproductive cycle, ie from the day of weaning up to the day of weaning of the next reproductive cycle. Blood samples were collected from each sow on the day of the study commencement , on the 30^{th} and 90^{th} days of pregnancy, at parturition and at weaning. Serum parameters measured included vitamins A and E, inorganic phosphorus, potassium, copper and zinc. Levels of all were found to be adequate throughout the reproductive cycle, verifying the safety of clinoptilolite in the diet of sows, although the level of vitamin E was found to be slightly lower in the experimental group.

Local Tolerance

Local tolerance was evaluated to ascertain whether clinoptilolite is tolerated at the sites in the body which may come into contact with the product as a result of its administration (Pavelic et al, 2001). No dermal toxicity or allergenicity was observed.

Repeated Dose Dermal Toxicity

Repeated dose dermal tolerance testing was performed on male Wistar rats and male BALB/c mice (Pavelic et al, 2001). Clinoptilolite was applied on the shaved skin of the whole dorsal region of animals in three ways: (a) as powder, (b) mixed with neutral cream in a ratio of 1:1, (c) mixed with paraffin oil at a ratio of 1:1. The animals were treated twice a day for 28 days. Macroscopic changes in the treated skin were examined daily. The left dorsal region of the animal was used as a control. For microscopic analysis of possible changes skin samples were collected one day after the last treatment. No dermal toxicity or allergenicity was observed.

Results and Discussion

Oral administration of clinoptilolite to mice and rats for 6 and 12 months, respectively, caused no changes that could be considered a toxic effect of treatment. The clinoptilolite shortened the prepregnancy period. The number of pups per litter was increased in clinoptilolite-treated mice, and probably for this reason the gain in body weight until weaning was decreased and a higher rate of mortality between days 8 and 21 of the neonatal period was observed. However, there are no differences between control and treated animals that would suggest reproductive toxicity attributable to the clinoptilolite administration. The clinoptilolite does not exhibit toxicity during organogenesis. The clinoptilolite did not exhibit dermal toxicity or allergenicity.

ANNEX: TOXICOLOGY OF ZEOLITE A (A SYNTHETIC SODIUM ALUMINIUM SILICATE)

Introduction

Zeolite A is a synthetic sodium aluminium silicate with empirical formula $Na_{12}Si_{12}Al_{12}O_{48}.27H_2O$ (Aarts et al, 2002; Thomas and Ballantyne, 1992). It is commonly used in household detergents to decrease water hardness by exchanging calcium ions for sodium ions (Aarts et al, 2002). Like clinoptilolite, Zeolite A has a cage-like structure consisting of SiO_4 and AlO_4 tetrahedra joined by shared oxygen atoms. However, Zeolite A has an Si:Al ratio of 1:1, compared with a ratio of >4:1 in clinoptilolite. Additionally, being of synthetic origin, Zeolite A contains only one type of cation. In other respects, Zeolite A and clinoptilolite are very similar. Numerous studies have been undertaken to examine the toxicity of Zeolite A (Aarts et al, 2002, and references therein). A review of the toxicology of Zeolite A has therefore been added as an annex to the dossier.

Neither developmental nor carcinogenic effects have been observed in any of the experimental studies on Zeolite A. No studies have been identified that investigated the reproductive toxicity of sodium aluminium silicate. However, no indications of toxicity to reproductive organs have been observed in long-term studies and no structure activity relationship is known that indicates a concern.

Toxicology Studies

Acute Oral Toxicity

The acute oral toxicity of sodium aluminium silicates has been investigated in several studies with rats, one study with mice and one with dogs (these have been reviewed by Aarts et al, 2002). In the rat studies, the administered doses ranged from 5,000 to 31,800 mg/kg body weight. The mouse study was conducted at 10,000 mg/kg body weight and the dog study at 1,000 mg/kg body weight. No mortality was observed in any of these studies.

Due to lack of acute toxicity it was not possible to determine an LD50 value in the studies conducted. The acute oral toxicity of sodium aluminium silicates is considered as very low.

Repeated Dose Toxicity

During the mid 1970's, repeated dose toxicity was studied in mice and rats in Henkel's laboratories (Aarts et al, 2002, and references therein). These trials are summarised below:

An unspecified sodium aluminium silicate was administered for 14 consecutive days to groups of male and female Fischer-344 rats (5 animals per group) and B6C3F1 mice (5 animals per group) in concentrations of 0, 0.625%, 1.25%, 2.5%, 5% and

10% (w/w) in the diet (Aarts et al, 2002). Based on body weight, food consumption and gross necropsy findings no marked signs of toxicity were observed.

Zeolite A was administered for 90 consecutive days to groups of male and female Wistar rats (20 animals per group) in concentrations of 0, 1,000ppm, 5,000ppm, 10,000ppm (w/w) in the diet (Aarts et al, 2002)). In the high dose group, urinary calculi were found in the bladders. Histological examination showed a hyperplastic reaction of the transitional epithelium in rats with calculi. No significant difference in the copper content of the livers was found between the control and high dose animals. However, the silicate content of the kidneys of male high dose animals was significantly elevated. The No Observed Adverse Effects Limit (NOAEL) was determined to be 5,000 ppm, which can be estimated to equal approximately 250 to 300 mg/kg/day.

An unspecified sodium aluminium silicate was administered for 163 days to groups of male and female COX-SD rats (20 animals per group) in concentrations of 0, 0.5%, 1.0%, 2.0% (w/w) in the diet (Aarts et al, 2002). Interim sacrifices were performed at 28 and 91 days. The 28 day sacrifice did not reveal any indication of test compound related toxicity. At 91 days, one animal at the high dose level was found to have bladder stones. Two others which died on days 84 and 85, respectively, showed evidence of bladder toxicity. During the extension of the study to 163 days, bladder stones appeared at the intermediate and low dose levels (one animal at each level) and more appeared at the high dose level. A NOAEL could not be deduced from this study.

The potential urogenital toxic effect was examined in a follow-up study in three groups of COX-SD rats (40 animals per group) each fed a diet with 0%, 0.125% or 2% (corresponding to approx. 69 and 1100 mg/kg body weight, respectively) of a sodium aluminium silicate (Zeolite A-type) for 160 or 200 days (Aarts et al, 2002). In the urine collections of treated rats white crystalline material was visible. A significant increase in the incidence of bladder and kidney stones was observed in the high dose group. Other than this there was no evidence of an alteration of urine parameters or kidney function. Pathological examination found histological changes of the kidneys and bladders in the high dose group only. In the kidneys, an increase in the severity of interstitial nephritis, regenerative epithelium and pelvic epithelial hyperplasia was reported, as was the frequent presence of a non-staining crystalline material in the pelvis of the kidneys. Also, the urinary bladders of these animals showed an increase in the incidence and severity of transitional epithelial hyperplasia. A NOAEL of 0.125% (approx 69 mg/kg body weight/day) is deduced from this study.

In a 24 week oral toxicity study, Long-Evans rats (10 males and 10 females per dose group) were fed a diet with 0, 0.125, 0.5 or 2% of Zeolite A-type sodium aluminium silicate (Aarts et al, 2002). Evaluation of mortality, physical appearance, feed efficiency, body weights, organ weights and organ / body weight ratios did not reveal evidence of any toxic effects at any of the dose levels. In the male and female rats of the intermediate and high dose groups, pathology revealed compound related microscopic alterations in the kidneys. The low dose diet did not result in any compound-related microscopic changes. The NOAEL in this study can therefore be considered to be 0.125% in the diet (approx 69 mg/kg body weight/day).

In an oral chronic toxicity study male and female Wistar rats were fed 0, 10, 100 and 1,000 ppm of sodium aluminium silicate of the Zeolite A-type (approx. equivalent to 0.6, 6.0 or 60 mg/kg/day) in the diet for 104 weeks (50 animals per dose group and sex) (Aarts et al, 2002). Mortality, feed consumption, body weights and water consumption were monitored. Ophthalmologic, blood, urinary and biochemical parameters were evaluated. After 104 weeks all animals were sacrificed. All organs were evaluated microscopically and macroscopically. No treatment-related signs of toxicity were observed and no indication of a chronic toxic response in any of the evaluated parameters was noted. No significant treatment-related effects were observed in any of the organs examined histopathologically. No treatment-related effect on the types or incidences of any neoplastic changes was observed. In this study the NOEL was therefore determined to be 60 mg/kg/body weight/day.

In a particularly interesting study, varying amounts (0%, 0.66%, 1.32% or 2.00%) of Zeolite A were fed to Quarter Horses (53 animals) commencing at six months of age (Nielsen et al, 1993). The horses were placed in race training at 18 months of age and fed diets containing 0%, 0.92%, 1.86%, or 2.8% Zeolite A. The treated animals were found to have increased plasma silicon concentrations and faster race times than the control group. Since the treatment groups receiving the two larger amounts of Zeolite A were worked greater distances than the control group before being injured, and since the medium treatment group completed more cycles before being injured than the control group, there is an indication that Zeolite A may be beneficial in preventing racing related injuries. A correlation between plasma silicon concentration and the distance travelled before injury in the group of horses which appeared more prone to injury is another indicator that Zeolite A may help prevent injury by providing bioavailable silicon to the horse. No toxic effects were reported.

Genetic Toxicity

The genetic toxicity of sodium aluminium silicate has been the subject of many in vitro and in vivo studies (Simmon and Eckford, 1979; Aarts et al, 2002).

Sodium aluminium silicate has been tested in Ames tests with Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 (Simmon and Eckford, 1979). No mutagenic potential was detected in these blind and independent studies.

A reverse mutation assay was conducted in Escherichia coli WP2 with sodium aluminium silicate with and without metabolic activation. No mutagenic potential was detected in this blind and independent study (Simmon and Eckford, 1979).

In a study in which human embryonic lung cell cultures were cultivated in the presence of different concentrations of sodium aluminium silicate, no clastogenic potential was observed (Aarts et al, 2002).

Male Albino rats (10-12 weeks old, 15 animals per group) were used in two sets of experiments with differing dosages for the evaluation of cytogenetic effects of sodium aluminium silicate in vivo. In both sets, a single dosing as well as repeated

dosing (5 consecutive days) was employed (Aarts et al, 2002). Triethylene melamine was used as a positive control and saline was used as a negative control. In the first set 4.25, 42.5 and 425 mg/kg body weight were administered orally by intubation. In the second set 5000 mg/kg body weight was administered. Observation time points were 6, 24 and 48 hours after dosing. Metaphase chromosome spreads were prepared from the bone marrow and scored for chromosomal aberrations. Neither the variety nor the number of chromosomal aberrations in bone marrow from dosed animals differed significantly from the negative controls. A positive response was observed in bone marrow from animals treated with triethylene melamine. The test compound was considered as non-mutagenic as measured by this assay.

In two further sets of experiments, similar treatment regimes were used to evaluate chromosomal aberrations of germ cells in the dominant lethal assay (10 animals per group) (Aarts et al, 2002). In these studies, following treatment, the males were mated to two females per week for eight weeks (seven weeks in the subacute study). Pregnant females were sacrificed at 14 days after separation from the male. At necropsy, the uterus was examined for early deaths, late foetal deaths and total implantations.

In the acute study of the first set, a significant (not dose dependent) decrease in average corpora lutea and preimplantation losses were seen in the experimental groups from mating weeks 4 and 5 when compared to the negative controls, but not when compared to the historical controls (Aarts et al, 2002). Average resorptions showed significant (but not dose dependent) increases in the experimental group from mating week 3 in all dose groups when compared to the negative control (zero value), but not when compared to historical controls. In the acute study using 5000 mg/kg body weight, no significant difference between negative control and the dosed animals was observed.

In the subacute study of the first set, significant dose-related increases (intermediate and high dose) in average implantations and corpora lutea were seen in the experimental groups from mating week 4 when compared to the negative control (Aarts et al, 2002). When compared to the historical controls, the negative as well as the intermediate dose group were significantly different. Significant dose-related increases in average resorptions were seen in the intermediate and high dose groups from mating week 6 when compared to the negative controls. However, no differences were observed when these groups were compared with the historical controls. In the subacute study using 5000 mg/kg body weight, a significant increase in preimplantation loss was observed in animals from mating week 1 and 3. This increase was attributed by the authors to a high number of corpora lutea unmatched by implantations in some females and was not regarded as compound related.

The positive control caused significant preimplantation loss and embryo resorption in animals from the first 5 mating weeks.

The authors concluded that the test compound does not induce dominant lethal mutations as measured by this study. They based their conclusion on the fact that no dose response or time trend patterns were revealed in the assay.

Male ICR mice (10 animals per group) were used in two sets of experiments with differing dosages of sodium aluminium silicate for the evaluation of gene mutations in the host mediated assay using ip injections of Salmonella typhimurium TA1530 and G46 as well as Saccharomyces cerevisiae (Aarts et al, 2002). In both sets, a single dosing as well as repeated dosing (5 consecutive days) was employed. The positive control was run by the acute system only using dimethyl nitrosamine for Salmonella and ethyl methane sulphonate for yeast, respectively. In the first set 4.25, 42.5 and 425 mg/kg body weight were administered orally by intubation. In the second set 5000 mg/kg body weight was administered. Three hours after administration of the test compound and indicator organism each animal was sacrificed. The indicator organisms were collected from the peritoneal cavity and the number of mutants was counted after plating on minimal agar. The test compound caused no significant increases in mutant or recombinant frequencies in both sets of experiments and in all doses used. No indication of genetic activity was detected in the host-mediated assay.

Carcinogenicity

An oral chronic toxicity study has been performed by Henkel (Aarts et al, 2002). Male and female Wistar rats were fed 0, 10, 100 and 1000 ppm of sodium aluminium silicate of the Zeolite A- type (approx equivalent to 0.6, 6.0 or 60 mg/kg/day) in their diet for 104 weeks (50 animals per dose group and sex). No treatment-related effect on the types or incidences of any neoplastic changes was observed in this study.

Reproductive Toxicity

No studies have been identified that investigated the reproductive toxicity of sodium aluminium silicate. However, no indication of toxicity to reproductive organs have been observed in long term studies and no structure activity relationship is known that indicates a concern.

Developmental Toxicity

Developmental toxicity has been studied in Charles River rats, Wistar rats, CD-1 mice, Dutch rabbits, New Zealand rabbits and Syrian hamsters. These trials are summarised below (Aarts et al, 2002):

Charles River rats were treated daily with sodium aluminium silicate with 0, 74 or 1600 mg/kg body weight on gestation days 6 – 15 (20 animals per dose). The dams were sacrificed on gestation day 20. Conception rates were high and no maternal, embryo or foetal toxicity was noted.

Wistar rats were treated daily with sodium aluminium silicate with 0, 16, 74, 345 or 1600 mg/kg body weight on gestation days 6-15. The dams were sacrificed on gestation day 20. The administration of the test compound had no clearly discernible effect on implantation or on maternal or foetal survival.

CD-1 mice were treated daily with sodium aluminium silicate with 0, 16, 74, 345 or 1600 mg/kg body weight on gestation days 6-15. The dams were sacrificed on gestation day 17. The administration of the test compound had no clearly discernible effect on implantation or on maternal or foetal survival.

Dutch rabbits were treated daily with sodium aluminium silicate with 0, 16, 345 or 1600 mg/kg body weight on gestation days 6-18. The dams were sacrificed on gestation day 29. The administration of the test compound had no clearly discernible effect on implantation or on maternal or foetal survival.

New Zealand rabbits were treated daily with sodium aluminium silicate with 0, 74, 345 or 1600 mg/kg body weight on gestation days 6-18 (20 animals per dose). The dams were sacrificed on gestation day 29.

Syrian hamsters were treated daily with sodium aluminium silicate with 0, 16, 74, 345 or 1600 mg/kg body weight on gestation days 6-10. The dams were sacrificed on gestation day 14. The administration of the test compound had no clearly discernible effect on implantation or on maternal or foetal survival.

In none of the above studies did the number of abnormalities seen in either soft or skeletal tissues in the test groups differ from the number occurring spontaneously in the control groups. This data shows that the sodium aluminium silicate was not teratogenic in the animals at the dose levels tested. In each study, the NOAEL was 1600 mg/kg body weight for maternal toxicity and for teratogenicity.

Biokinetics

In an experiment to study the biokinetics of ingested sodium aluminium silicate, five male Wistar rats were given an oral dose of 1000 mg/kg of Zeolite A (Aarts et al, 2002). Urine and faeces were sampled over 24 hours. The results showed that about 1% of the silicon administered orally was absorbed and eliminated via the kidney. The aluminium balance indicated that the absorption of this component is poor. The majority of the administered Zeolite A was eliminated via the faeces, as was the silica. Analysis of organs for silicon did not indicate an accumulation of sodium aluminium silicate after oral administration.

The rate of urinary excretion of silicon and aluminium was determined in a group of adult male Sprague-Dawley Cox rats (4 rats per group) after single oral administration of Zeolite A. The doses were 0, 40, 200 or 1000 mg/kg body weight. Urine was collected in periods of 0-24, 24-48, 48-72 and 72-96 hours after dosing and was analysed for silicon and aluminium by induction-coupled RF plasma optical emission spectrometry. The amount of silicon excreted within 96 hours increased with increasing dose showing saturation kinetics (about 200ì g at low doses about 800ì g at intermediate dose, and about 1400ì g at high dose levels). A half life of 6-8 hours was determined. There was no detectable increase in the aluminium content of the urine. The authors postulated that Zeolite A is hydrolysed in the gastrointestinal tract, and that from the resulting breakdown products only the silicon species is absorbable. Since the half life was not dose dependent and taking into

account additional data on other silicates investigated by the authors, they concluded that hydrolysis (a prerequisite of silicon species absorption) is the rate limiting step

The bioavailability of silicon and aluminium from sodium zeolite A has been studied in beagles, using doses of 30 mg/kg administered as a capsule, oral suspension or oral solution, relative to an intravenous bolus solution (Cefali et al, 1995 & Cefali et al, 1996). Twelve dogs received single doses of zeolite A after a 1 week control period in a randomised five-way crossover design. Plasma samples were drawn at time 0 and for 36 hours after dosing. The concentrations of silicon and aluminium were determined by graphite furnace atomic absorption. The bioavailability of silicon was determined to be 2.33, 3.44 and 2.73% for the capsules, the oral suspension and the oral solution respectively. The mean half life was 17.5 hours. The bioavailability of aluminium was determined to be less than 0.1%, with a mean half life of 91.2 hours.

Human Exposure in Industry

Workers in a production plant and laboratory have been examined over a period of 15 years (IUCLID Dataset 1344-00-9, 2000). The average number of employees examined was 100 per year. Most of these employees were examined as many as 10 to 15 times. Examination included a complete history, physical, chest X-ray and urinalysis. No evidence of systemic, generalised or local reactions due to sodium aluminium silicate have been found.

Results and Discussion

The only observed adverse effects after oral ingestion of sodium aluminium silicates were those seen in the kidney and urinary bladder. These effects have been consistently reported in the repeated dose toxicity studies. In one study especially designed to investigate the effects of sodium aluminium silicates on the urogenital tract, microscopic changes in the kidney and bladder were found, associated with crystalline material excreted in the urine. No other differences in urinary parameters were observed. These findings may be explained by absorption of small amounts of silicon compounds from the gastrointestinal tract after dissociation of the sodium aluminium silicate to sodium, aluminium and SiO_4. The concentration of SiO_4 in the kidney, the subsequent formation of crystalline material and the excretion of this material via the urine may cause mechanical damage to the kidney and bladder, leading to epithelial hyperplasia in these organs. The NOAEL for these effects was determined as 69 mg/kg body weight/ day in a 200 days study. A chronic study of 104 weeks duration did not show any toxic effects at the highest dose (60 mg/kg body weight / day) and corroborated the NOAEL for rats observed in the 200 day study.

In vitro test systems did not detect any genetic toxicity of sodium aluminium silicates. These results were corroborated by in vivo test systems. In a cytogenetic assay in rats, a dominant lethal assay in rats, and a host-mediated assay in mice, in which doses ranged from 4.25 to 5000 mg/kg body weight and acute and subacute dosing regimes employed, no indication of genetic toxicity of the test compound was found.

The long term oral (and inhalation) studies did not find any potential of sodium aluminium silicate to induce neoplastic lesions. Tests also failed to reveal any potential of sodium aluminium silicate to induce silicotic reactions in the experimental animals.

Sodium aluminium silicate has been evaluated for teratogenicity in rats, mice, rabbits and hamsters. No teratogenic effects were observed in experimental animals. The NOAEL in the studies performed was 1600 mg/kg body weight for both maternal toxicity and teratogenicity.

Ingested sodium aluminium silicate is mostly excreted in the faeces. However, a small but significant proportion is hydrolysed in the digestive tract and a silicon based compound is absorbed and excreted in the urine. Most of the absorbed silicon is excreted within 24 hours of administration; in rats the half life has been found to be 6-8 hours. About 12% of the administered silicon dose is absorbed at doses between 40 and 200 mg/kg body weight in rats. The aluminium component of the parent compound is absorbed only to an extent of less than 0.1% of the administered dose. The low absorption rate for the aluminium component has also been observed in beagles. In this species, the oral bioavailability for the silicon compound was lower than observed in the rat.

To summarise, sodium aluminium silicate has a very low toxicity after oral (or dermal) application. The LD50 is higher than 5000 mg/kg body weight in experimental animals. A range of in vitro and in vivo studies on genetic toxicity found no potential of sodium aluminium silicate to induce neoplastic lesions. Chronic oral studies demonstrate that sodium aluminium silicate causes adverse effects in the urogenital tract at high doses. Studies on reproductive toxicity are not available. The data on developmental toxicity demonstrate that sodium aluminium silicate is not teratogenic in experimental animals.

Conclusions

Both Clinoptilolite and Zeolite A have been extensively tested for toxicity in a wide range of animals, including rats, mice, hamsters, beagles and pigs. Acute and chronic oral toxicity, developmental toxicity, genetic toxicity, carcinogenicity and biokinetics have been studied. Both Clinoptilolite and Zeolite A appear to lack toxic effects unless ingested in very large quantities, in which case white crystals of a break-down product appear in the kidneys and urogenital tract, with associated microscopic changes. Significantly, clinoptilolite has been approved by the Cuban Drug Control Agency for use in the anti-diarrheic drug ‘Enterex’. Workers in a production plant handling Zeolite A have been monitored for a period of fifteen years, with no adverse effects having been reported. No fatal case arising from the oral uptake of either Clinoptilolite or Zeolite A has been identified.

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