SCIENCE and TECHNOLOGY

NATURAL EXTRACTS RESEARCH PTY LTD

ABN: 39-079-114-202

PRODUCT SAFETY AND TOXICOLOGY

One of the most important pieces of information for both the medical practitioners, their patients and all other users, is to know that the product is safe and non-toxic. In this document we have presented numerous studies and abstracts from research papers that show that the active ingredient is safe and non-toxic. In fact after reading the information contained herein, it would appear that the product is actually beneficial to the body even when not under medical treatment.

1 Australian Sourced Unpolluted Alumino-Silicate

ZeoActiv8™ is a proprietary formula with its active ingredient being pure Australian sodium/calcium alumino-silicate or 'zeolite' (the type of alumino-silicate used is called 'clinoptilolite') The zeolite used by Natural Extracts Australia Pty. Ltd. (the 'Company') is extracted from a mine in Australia which has an unusually pure source of clinoptilolite. The deposit was only discovered and geologically mapped just over twelve years ago. There are very few deposits in the world of this purity because it comes from an area away from any major city or industrial complex. There is little or no airborne or water pollution to contaminate it. It has virtually no other foreign materials (such as clays, gypsum, erionite etc.) within it. That is why this Australian zeolite is recognised as one of the world's purest and therefore one of the most effective.

2 Particle Shape

Clinoptilolite is a sheet zeolite which is considered completely safe and non-toxic because its crystals tend to be rounded. Overseas and Australian scientific research, over a long period of time, has indicated that these types of zeolites cause no internal damage if they are ingested or inhaled

Fig.6 Coarser zeolite grains. SEM. Magnif. 263X

Above is a electron microscope photo of the zeolite used by the Company in its products showing the sub-micronised particles are rounded which eliminates the possibility of internal wounding to the digestive system. There are articles reaffirming this conclusion presented in this document. The

zeolite is mined and the put through a proprietary manufacturing process to reduce its average size to approximately 800 nanometers (.8 of a micron). The small particles have no trouble passing safely through the intestinal system.

After it is mined the raw material is micronised and washed twice in solutions to remove any impurities. The solutions are non acid based and do not leave behind a residue. The material is then heated to remove any bacteria, washed in special proprietary solutions and sealed in air tight containers ready for use.

3 Particle Size

The particle size report below was done using a Malvern Hydro 2000MU laser particle size scanner. It shows, [where the two red lines intersect], that approximately 80% of the particles, [the numbers on the right hand side of the chart], are below one micron [the numbers on the bottom of the chart].

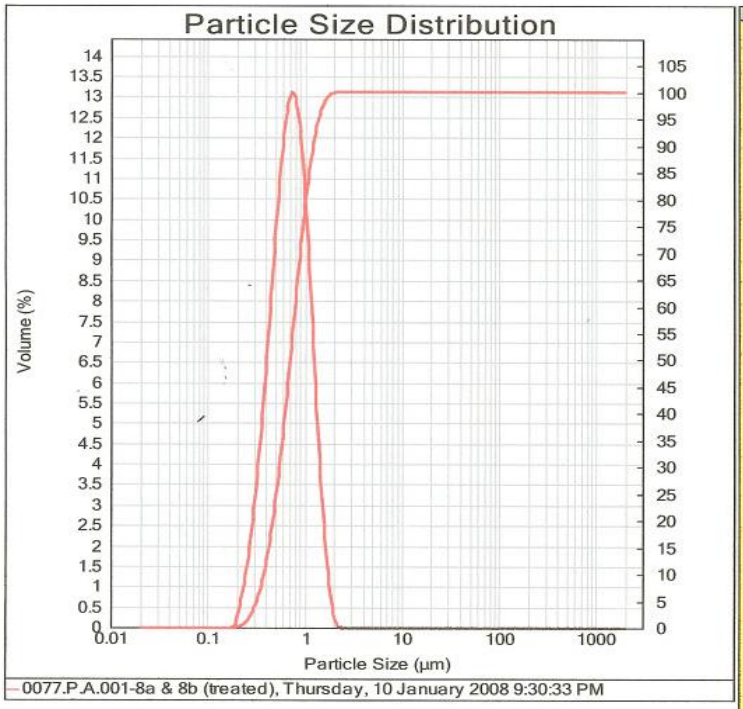
Malvern Hydro2000MU Laser Particle Sizing

Services Requisition:	0077.P.A.001	**Supplier:**	Zeolite
Sample ID:	0077.P.A.001-8a & 8b (treated)	**Measured by:**	ouo
Date:	Thursday, 10 January 2008 9:30:33 PM	**Analysis model:**	General purpose

Particle Name:	Fraunhofer	**Particle RI:**	0.000
Dispersant Name:	Water	**Dispersant RI:**	1.330
Absorption:	0	**Obscuration:**	16.82

Vol. Weighted Mean D[4,3]:	0.759	um	**Result units:**	Volume	
Surface Weighted Mean D[3,2]:	0.625	um	**Specific Surface Area:**	9.61	m2/g

D(0.10) : 0.38 µm D(0.50) : 0.70 µm D(0.80) : 1.02 µm D(0.90) : 1.22 µm
D(0.97) : 1.50 µm D(0.98) : 1.58 µm D(0.99) : 1.70 µm D(1.00) : 2.26 µm



Size (µm)	Vol Under %	Size (µm)	Vol Under %
0.010	0.00	11.482	100.00
0.011	0.00	13.183	100.00
0.013	0.00	15.136	100.00
0.015	0.00	17.378	100.00
0.017	0.00	19.953	100.00
0.020	0.00	22.909	100.00
0.023	0.00	26.303	100.00
0.026	0.00	30.200	100.00
0.030	0.00	34.674	100.00
0.035	0.00	39.811	100.00
0.040	0.00	45.709	100.00
0.046	0.00	52.481	100.00
0.052	0.00	60.256	100.00
0.060	0.00	69.183	100.00
0.069	0.00	79.433	100.00
0.079	0.00	91.201	100.00
0.091	0.00	104.713	100.00
0.105	0.00	120.226	100.00
0.120	0.00	138.038	100.00
0.138	0.00	158.489	100.00
0.158	0.00	181.970	100.00
0.182	0.00	208.930	100.00
0.209	0.10	239.883	100.00
0.240	0.77	275.423	100.00
0.275	2.18	316.228	100.00
0.316	4.86	363.078	100.00
0.363	8.49	416.869	100.00
0.417	13.93	478.630	100.00
0.479	21.19	549.541	100.00
0.550	30.23	630.957	100.00
0.631	40.63	724.436	100.00
0.724	52.44	831.764	100.00
0.832	64.23	954.993	100.00
0.955	75.23	1096.478	100.00
1.096	84.53	1258.925	100.00
1.259	91.56	1445.440	100.00
1.445	96.19	1659.587	100.00
1.660	98.79	1905.461	100.00
1.905	99.61	2187.762	100.00
2.188	100.00	2511.886	100.00
2.512	100.00	2884.032	100.00
2.884	100.00	3311.311	100.00
3.311	100.00	3801.894	100.00
3.802	100.00	4365.158	100.00
4.365	100.00	5011.872	100.00
5.012	100.00	5754.399	100.00
5.754	100.00	6606.934	100.00
6.607	100.00	7585.776	100.00
7.586	100.00	8709.636	100.00
8.710	100.00	10000.000	100.00
10.000	100.00		

Operator notes: *0077.P.A.001-8a & 8b (treated)*

Malvern Instruments Ltd.
Malvern, UK

Mastersizer 2000 Ver. 5.11
Serial Number : 34027-37

File name: 0077.P.A.001-8.mea
Record Number: 22

4 Tests and Research Reports to Show the Active Ingredient's Low Toxicity

There have been many studies on the safety of the zeolite, clinoptilolite. It is considered safe and has GRAS (generally recognized as safe) status from the FDA in the United States. More than thirty five studies have been summarised to support our statement that the active ingredient is safe.

1 In the United Kingdom the company, Euremica Environmental Ltd, of Instrument House, Morgan Drive, Guisborough, Cleveland TS14 7DG United Kingdom made an Application for the Approval of Clinoptilolite to be classified as a food 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.*

Euremica Environmental Ltd ('Euremica') had proposed to market clinoptilolite in capsules as a human food supplement. The clinoptilolite was also to be sourced from Australia and Euremica had carried out a number of tests to ensure its suitability as a food source [pages 6 to 13 of the report].

As we understand the application was refused as a food supplement because it did not comply with the pharmaceutical regulations of the European Parliament. However the non toxicity information contained therein is very applicable to the clinoptilolite used in the Company's product. They are both Australian zeolites (see below) with the same structure and are nearly identical. [a full copy of the application document is on file in our scientific compendium.

In addition the application refers and draws inference many times from the HERA report supposedly of Aarts et al. This report was actually prepared by Henkel KGaA, Düsseldorf with the assistance of the members of the HERA Environmental Task Force and the HERA Human Health Task Force of which Aarts was just one member but first on the alphabetical list of members with two a's in the surname.

The HERA report is an in-depth study of the use of a synthetic zeolite, referred to as Zeolite 'A' This product is used in washing powders and the report was conducted to assess whether there was any possibility of any consumer health or environmental exposure or contamination problems that may have resulted from Zeolite A's use. The report was completed for the European Parliament. [The complete report has been also included in appendix B and is [59] on the master reference list of the compendium].

The introduction to the report follows

"Introduction

[244] *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 SiO4 and AlO4 tetrahedra joined by shared oxygen atoms (Mumpton, 1983). The negative charges of the AlO4 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 moulds 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 feeding stuffs 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', [72] 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."

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 in 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.'

As the safety and toxicology information is extremely important and very relevant we have set it out here in full being pages [33 to 40] of their original application.

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

Introduction
Zeolite A is a synthetic sodium aluminium silicate with empirical formula Na12Si12Al12O48.27H2O (Aarts et al, 2002[59]); 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 [59]). Like clinoptilolite, Zeolite A has a cage-like structure consisting of SiO4 and AlO4 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[59]), 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 [59]). 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[59]), 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 34 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 [59]). 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 [59]). 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 [59]). 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 [59]). 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 [59]). 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 NOAEL 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 bio-available 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 [59]). 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 [59]).

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 [59]). 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 nonmutagenic 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[59]). 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 [59]). 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 [59]). 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 historicalcontrols. 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 200ig at low doses about 800ig at intermediate dose, and about 1400ig 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 SiO4. The concentration of SiO4 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 brea k-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' [72]. 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.

The conclusions are self evident. From all the data available in the application by Euremica, clinoptilolite appears to be completely safe and non toxic unless ingested in very large quantities. All this information is based upon the HERA report into using zeolite A in washing powder. The complete report is available in our scientific compendium.

What other evidence is there available?

2 When two zeolites were placed in simulated body fluids ('SBF') for a period of 14 days no biological effects were observed except a small transfer of potassium.

[54] Abstract

Various zeolites were kept in simulated body fluid (SBF) for different periods of time. Possible changes that may occur in the crystalline structures of zeolites and the chemical composition of SBF were determined by various analysis techniques after this treatment. The possible effects of two different zeolites on the morphology and viability of chronic myelogeneous leukemia and swiss albino fibroblast culture cells were also investigated. It was determined that when different types of zeolites were kept in the SBF for up to 14 days, their crystal structures were not affected. Observable amounts of Si were detected in the SBF samples after their treatment with all the zeolites investigated. Another variation in the chemical composition of SBF, worth to mention, was the increase of about 10% in its K content after the treatment carried out by using clinoptilolite. The zeolites KA and silicalite, which allowed the lowest and highest amount of silicon transfer into the SBF, respectively, were observed not to have any significant biological effect on the two different cell generations investigated under the conditions used in this study.

3 Investigation of cytotoxicity of six mineral dusts from 12 deposits. Results showed that clinoptilolite and one other, being rounded in shape, had no AM cytotoxicity.

[53] Abstract

"In order to study the damage mechanism of mineral dusts on the pulmonary alveolar macrophages (AM), the changes of their death ratio, malandialdthyde (MDA) content and activities of lactate dehydrogenase (LDH) and superoxide dismutase (SOD) were measured. And the technique of cell culture in vitro was used to investigate the cytotoxicity of six mineral dusts (twelve crystal habits) from twelve mineral deposits. The results showed that wollstonite and clinoptilolite had no AM cytotoxicity while other fibrous and grainy mineral dusts could damage pulmonary AM in various degrees. The cytotoxicity of fibrous mineral dusts was greater than that of the grainy ones, and the cytotoxicity of dusts was positively correlated with the active OH- content in dusts, but not necessarily so with its SiO2 content. The high pH values produced by dust was unfavourable for the cells survival and the dusts with a low bio-resistance were safe for cells. The content of variable valence elements in dusts could influence their cytotoxicity and the surface charge of dusts was not a stable factor on their toxicity. It indicates that the shape of mineral dusts is one of the factors affecting cytotoxicity, and that the cytotoxicity of mineral dusts mainly depends on their properties."

4 This report discusses the safety of using a number of clays, earths and alumino silicates which are used in various cosmetic applications. Of the zeolites is said that they were not significantly toxic in oral acute or short term oral or parental studies in animals. Reference was made to inhalation toxicity. But these concerns relate only to large particle size, long, wide fibres which are not found in clinoptilolite.

Final report on the safety assessment of aluminium silicate, calcium silicate, magnesium aluminium silicate, magnesium silicate, magnesium trisilicate, sodium magnesium silicate, zirconium silicate attapulgite, bentonite, Fuller's earth, hectorite, kaolin, lithium magnesium silicate, lithium magnesium silicate, lithium magnesium sodium silicate, montmorillonite, pyrophyllite, and zeolite.

[52] Abstract

"This report reviews the safety of Aluminium, Calcium, Lithium Magnesium, Lithium Magnesium Sodium, Magnesium Aluminium, Magnesium, Sodium Magnesium, and Zirconium Silicates, Magnesium Trisilicate, Attapulgite, Bentonite, Fuller's Earth, Hectorite, Kaolin, Montmorillonite, Pyrophyllite, and Zeolite as used in cosmetic formulations. The common aspect of all these claylike ingredients is that they contain silicon, oxygen, and one or more metals.

Many silicates occur naturally and are mined; yet others are produced synthetically. Typical cosmetic uses of silicates include abrasive, opacifying agent, viscosity-increasing agent, anticaking agent, emulsion stabilizer, binder, and suspending agent. Clay silicates (silicates containing water in their structure) primarily function as adsorbents, opacifiers, and viscosity-increasing agents. Pyrophyllite is also used as a colorant. The International Agency for Research on Cancer has ruled Attapulgite fibers >5 micron as possibly carcinogenic to humans, but fibers <5 micron were not classified as to their carcinogenicity to humans.

Likewise, Clinoptilolite, Phillipsite, Mordenite, Nonfibrous Japanese Zeolite, and synthetic Zeolites were not classified as to their carcinogenicity to humans. These ingredients are not significantly toxic in oral acute or short-term oral or parenteral toxicity studies in animals. Inhalation toxicity, however, is readily demonstrated in animals. Particle size, fibrogenicity, concentration, and mineral composition had the greatest effect on toxicity. Larger particle size and longer and wider fibers cause more adverse effects.

Magnesium Aluminum Silicate was a weak primary skin irritant in rabbits and had no cumulative skin irritation in guinea pigs. No gross effects were reported in any of these studies. Sodium Magnesium Silicate had no primary skin irritation in rabbits and had no cumulative skin irritation in guinea pigs. Hectorite was nonirritating to the skin of rabbits in a Draize primary skin irritation study.

Magnesium Aluminium Silicate and Sodium Magnesium Silicate caused minimal eye irritation in a Draize eye irritation test. Bentonite caused severe iritis after injection into the anterior chamber of the eyes of rabbits and when injected intralamellarly, widespread corneal infiltrates and retrocorneal membranes were recorded. In a primary eye irritation study in rabbits, Hectorite was moderately irritating without washing and practically non irritating to the eye with a washout.

Rats tolerated a single dose of Zeolite A without any adverse reaction in the eye. Calcium Silicate had no discernible effect on nidation or on maternal or fetal survival in rabbits. Magnesium Aluminium Silicate had neither a teratogenic nor adverse effects on the mouse fetus. Female rats receiving a 20% Kaolin diet exhibited maternal anemia but no significant reduction in birth weight of the pups was recorded. Type A Zeolite produced no adverse effects on the dam, embryo, or fetus in either rats or rabbits at any dose level. Clinoptilolite had no effect on female rat reproductive performance. These ingredients were not genotoxic in the Ames bacterial test system.

In primary hepatocyte cultures, the addition of Attapulgite had no significant unscheduled DNA synthesis. Attapulgite did cause significant increases in unscheduled DNA synthesis in rat pleural mesothelial cells, but no significant increase in sister chromosome exchanges were seen. Zeolite particles (<10 micron) produced statistically significant increase in the percentage of aberrant metaphases in human peripheral blood lymphocytes and cells collected by peritoneal lavage from exposed mice.

Topical application of Magnesium Aluminium Silicate to human skin daily for 1 week produced no adverse effects. Occupational exposure to mineral dusts has been studied extensively. Fibrosis and pneumoconiosis have been documented in workers involved in the mining and processing of Aluminium Silicate, Calcium Silicate, Zirconium Silicate, Fuller's Earth, Kaolin, Montmorillonite, Pyrophyllite, and Zeolite.

The Cosmetic Ingredient Review (CIR. The Cosmetic Ingredient Review (CIR) Expert Panel concluded that the extensive pulmonary damage in humans was the result of direct occupational inhalation of the dusts and noted that lesions seen in animals were affected by particle size, fiber length, and concentration. The Panel considers that most of the formulations are not respirable and of the preparations that are respirable, the concentration of the ingredient is very low. Even so, the Panel considered that any spray containing these solids should be formulated to minimize their inhalation. With this admonition to the cosmetics industry, the CIR Expert Panel concluded that these ingredients are safe as currently used in cosmetic formulations. The Panel did note that the cosmetic ingredient, Talc, is a hydrated magnesium silicate.

Because it has a unique crystalline structure that differs from ingredients addressed in this safety assessment, Talc is not included in this report."

5 Various materials were introduced into the lungs of rats and the lung tissue examined after 90, 180 and 360 days. Clinoptilolite was found to be inert while all the others showed cytotoxic effects to some degree.

[2] Abstract [full paper Appendix M]

The effects of samples of crystalline quartz, diatomaceous earth, mordenite and clinoptilolite were investigated in vitro (as concerns erythrocyte haemolysis and lactate dehydrogenase (LDH) release from peritoneal macrophages) and in vivo (on LDH, protein and phospholipids in rat bronchoalveolar lavage (BAL), and phospholipids in rat lung tissue). The respirable mineral samples were instilled intratracheally. Determinations in the BAL were carried out after 15, 60 and 180 days, and in the lung tissue after 90, 180 and 360 days. Quartz DQ and quartz FQ induced acute, subacute and chronic inflammation and progressive fibrosis. However, due to the Al2O3 contamination on the surface of the particles quartz FQ caused a delayed response in vivo. Diatomaceous earth produced acute/subacute inflammation that gradually became more moderate after 60 days. Clinoptilolite was inert, whereas the other zeolite sample, mordenite, was cytotoxic in vivo. The reason for this was presumably the needle and rod-shaped particles in the mordenite samples. The investigation revealed that different in vitro and in vivo methods can provide valuable data concerning the pulmonary toxicity of minerals.

6 This study showed that clinoptilolite had no carcinogenic incidence of tumours in any tissues or organs and no carcinogenic activity in rats.

[8] Abstract [full paper Appendix M]

The long term carcinogenicity of clinoptilolite was examined in Wistar rats. Respirable clinoptilolite was given intratracheally in doses 0, 30, or 60 mg/animal to groups of 60 or 50 male and 50 female rats on one occasion. No significant dose-related increase was found in the incidence of tumours in any organ or tissue. The results indicated that clinoptilolite type zeolite had no carcinogenic activity in Wistar rats.

7 Rats intoxicated with dichlorvos showed that clinoptilolite gave a marked protective effect on most tissues studied.

[10] Abstract

In the first series of trials, the physiological values of tissue cholinesterases were determined in the male rats of the Wistar strain. In the second series of trials the rats were perorally intoxicated with dichlorvos at the doses of 200.0, 128.0, 81.9, 65.5 and 52.4 mg per kg live weight. The objective of the trials was to examine the distribution of dichlorvos in the body of a rat on the basis of tissue cholinesterase inhibition. A marked decrease in the level of tissue cholinesterases was recorded at all the dichlorvos doses. In the third series of trials the protective effect of clinoptilolite was verified; clinoptilolite as a sorbent of natural origin has been administered per os at the dose of 1.0 g per kg live weight just before the intoxication with dichlorvos. The results document a marked protective effect of clinoptilolite on most of the tissues studied.

8 Another test involving rats. These were intoxicated by VX substance and those which had been given clinoptilolite showed significantly lowered ChE than the control group.

[6] Abstract

*In the present paper the effect of zeolite tuff (61% clinoptilolite) was investigated on cholinesterase activity in brain, liver, spleen, femoral muscle, heart, stomach, duodenum, colon and erythrocytes in sewer-rats after peroral intoxication with VX substance (65.5 micrograms/kg). Fig. 1 shows the ChE activity in the tissues and erythrocytes in the animals of control group and in the group of animals after intoxication with VX substance. The highest activity in the control group was found in brain and duodenum. The enzyme activity in the femoral muscle had the lowest values. A significant decrease in the ChE activity ($P < 0.001$ or $P < 0.01$) occurred in all the investigated samples in the group of animals intoxicated with the VX substance. Highest enzyme inhibition was observed in erythrocytes (97.9%), stomach (97.9%), brain (95.4%) and liver (94.7%) if compared with the control group. The relatively lowest inhibition was found out in duodenum and colon. In the group administered zeolite before intoxication (1.0 g/kg five minutes before intoxication) the ChE activity was significantly higher in almost all investigated samples than in the group without zeolite ($P < 0.001$ or $P < 0.01$)-Fig. 2. The duodenum is an exception, in which the ChE activity in the zeolite group was lower than in the zeolite-free group ($P < 0.001$), as well as the colon, in which there were no significant differences in the activity between the groups.**(ABSTRACT TRUNCATED AT 250 WORDS)***

9 Clinoptilolite given to sheep contaminated with radiocaesium showed marked reduction in the amount of contamination.

[31] Abstract

The efficiency of the sorbent prepared by immobilization of [Iron(II)hexacyanoferrate(II)] on clinoptilolite--marked as ZEOFe--in reduction of the radiocaesium Cs-137 has been in vivo investigated in sheep. It was found that an application of this modified clinoptilolite affected both primary and secondary resorption of Cs-137 also by interrupting the enteral cycle of radiocaesium in sheep. It was proved that ZEOFe accelerated approx. twice the excretion of Cs-137 from sheep's body. The whole effect resulted in 15 to 50 times lowering of the equilibrium concentration of radiocaesium in the case of constant intake of the contaminated feed and simultaneous application of 50 grams of ZEOFe daily. The actual reduction depends mainly on the way of administration. The reduction of Cs-137 by non-modified clinoptilolite--ZEO--has been investigated, too. More than 10x lower sorption efficiency has been observed in comparison with ZEOFe.

10 Clinoptilolite has been approved for use as an anti-diarrheic drug. Enterex: Anti-diarrheic drug based on purified natural clinoptilolite

[72] Abstract

A new anti-diarrheic drug for humans has been developed based on the physical and chemical properties of the purified natural clinoptilolite from NZ. A series of physical, chemical, technological, pharmacological, microbiological, and clinical studies were successfully conducted to meet the requirements of the Cuban Drug Quality Agency. The most important results concerning the properties and biological mechanism of NZ zeolite are described in this paper.

11 Antiviral properties of clinoptilolite of this study suggest that there is a therapeutical application for clinoptilolite against the herpes virus.

[74] Abstract

The aim of this study was to evaluate the antiviral properties of clinoptilolite, a natural non-toxic zeolite. Herein, a fine powder of micronized zeolite (MZ) was obtained by tribomechanical micronization of natural clinoptilolite. Different viral suspensions were treated with MZ in concentrations ranging from 0.5 to 50 mg/ml. The viral proliferation was evaluated by optical microscope as percentage of cytopathic effect (CPE). Human adenovirus 5, herpes simplex virus type 1 (HSV 1) and human enteroviruses (coxsackievirus B5 and echovirus 7) were used in the antiviral assay. Concentrations of 0.5 and 5 mg/ml of MZ induced a very low antiviral effect or the antiviral was not observed at all, while concentrations of 12, 25 and 50 mg/ml of MZ induced a significant inhibitory effect upon viral proliferation. MZ inhibited the viral proliferation of HSV 1, coxsackievirus B5 and echovirus 7 more efficiently than adenovirus 5. The antiviral effect of MZ seems to be non-specific and is more likely based on the incorporation of viral particles into pores of MZ aggregates than ion exchange properties of clinoptilolite. Our preliminary results indicate a possibility of therapeutical application of MZ, either locally (skin) against herpes virus infections or orally in cases of adenovirus or enterovirus infections. Futhermore, MZ could also be used in purification of drinking water from different viruses.

12 The anticancer and antioxidative effects of micronized zeolite clinoptilolite showed a strong reduction of cancer count.

[56] Abstract

BACKGROUND: Treatment of cancer-bearing mice and dogs with micronized zeolite clinoptilolite (MZ) led to improvement of the overall health status, prolongation of life span and decrease of tumour size in some cases. It also reduced lipid peroxidation in the liver of mice. MATERIALS AND METHODS: The experiments were performed on various tumour cell cultures and tumour-bearing animals. Immunohistochemistry was used to analyse if MZ could interfere with Doxorubicin-induced lipid peroxidation and consequential production of 4-hydroxynonenal (HNE). RESULTS: MZ reduced the metabolic rate of cancer cells and increased binding of HNE to albumin in vitro. It selectively reduced generation of HNE in vivo in tumour stroma after Doxorubicin treatment leaving onset of lipid peroxidation intact in malignant cells. Combined treatment with Doxorubicin and MZ resulted in strong reduction of the pulmonary metastasis count increasing anticancer effects of Doxorubicin. CONCLUSION: Interference of MZ with lipid peroxidation might explain some of the beneficial effects of this particular zeolite in combined cancer therapy.

13 The effect of natural clinoptilolite on the serotonergic receptors in the brain of mice showed that there was a beneficial effect on those mice with mammary carcinoma.

[1] Abstract

The ex vivo effect of tribomechanically micronized zeolite (MZ) on the binding of 3H-8-OH-DPAT to 5-HT(1A) and 3H-5-HT to 5-HT(1B) receptors was investigated in the brain of non-tumourous (control) and mammary carcinoma bearing female mice. During 14 and 28 days mice were fed with standard food, standard food supplemented with 25% of MZ, or standard food supplemented with 25% of non tribomechanically micronized zeolite (non-MZ). A reduced binding of 3H-8-OH-DPAT to 5-HT(1A) receptors in mammary carcinoma bearing mice was found when compared to control mice fed with standard food for 28 days, suggesting a time dependent alteration of 5-HT(1A) receptors in mammary carcinoma. The addition of MZ for 28 days in these mice abolished the decrease in 5-HT(1A) receptors binding, indicating a possible beneficial effect of MZ, at least on 5-HT(1A) receptors in mammary carcinoma bearing mice. The preliminary data show that MZ administered as a food supplement (25%) for 14 days induced a transient decrease in the binding of 3H-5-HT to brain 5-HT(1B) receptors only in control, but not in tumour-bearing mice, that disappeared after 28 days of MZ-supplemented food administration. The mechanism of the indirect action of MZ on the brain serotonergic receptors might be achieved by the alterations in the electrolytes balance, and/or by the regulation of the immune system.

14 Dietary supplementation with an activated zeolite clinoptilolite in immunodeficiency: effects on the immune system which showed no adverse reactions to the treatment.

[67] Abstract

Natural zeolites are crystalline aluminosilicates with unique adsorption, cation-exchange, and catalytic properties that have multiple uses in industry and agriculture. TMAZ, a natural zeolite clinoptilolite with enhanced physicochemical properties, is the basis of the dietary supplements Megamin and Lycopenomin, which have demonstrated antioxidant activity in humans. The aim of this prospective, open, and controlled parallel-group study was to investigate the effects of supplementation with TMAZ on the cellular immune system in patients undergoing treatment for immunodeficiency disorder. A total of 61 patients were administered daily TMAZ doses of 1.2 g (Lycopenomin) and 3.6 g (Megamin) for 6 to 8 weeks, during which the patients' primary medical therapy was continued unchanged. Blood and lymphocyte counts were performed at baseline and at the end of the study. Blood count parameters were not relevantly affected in either of the two treatment groups. Megamin administration resulted in significantly increased CD4+, CD19+, and HLA-DR+ lymphocyte counts and a significantly decreased CD56+ cell count. Lycopenomin was associated with an increased CD3+ cell count and a decreased CD56+ lymphocyte count. No adverse reactions to the treatments were observed.

15 Clinoptilolite effects the cellular micro-environment of cell media and consequently, has an effect on tumour cells in vitro.

[55] Abstract

Clinoptilolite is a nontoxic natural zeolite with properties of an ion-exchanger and adsorbent. Earlier studies showed that clinoptilolite could be an adjuvant in cancer therapy. The aim of this study was to define effects of clinoptilolite in cell media on cell viability and activity of key proteins regulating cell survival, cell division and stress response. The number of viable cells, DNA synthesis and activity of EGF-R, PKB/Akt and NF?B was reduced, while apoptosis was increased in cells that were cultured in medium supplemented with clinoptilolite. These results might be due to adsorption of some serum components such as EGF to clinoptilolite. In treated medium without serum the predominant role of clinoptilolite is that of cation exchange, likely affecting calcium levels and calcium-dependent signalling pathways. These results are in line with other data that confirm enhanced apoptosis in cells incubated in treated medium. Together, data presented here demonstrate that clinoptilolite affects cellular microenvironment through mechanisms that are dependent on adsorptive and ion-exchange characteristics of this material

16 Mice and dogs suffering from cancer tumours had improved health and longer life span after clinoptilolite powder was used to treat their tumours. The treatment showed no negative effects.

[58] Abstract

Natural silicate materials, including zeolite clinoptilolite, have been shown to exhibit diverse biological activities and have been used successfully as a vaccine adjuvant and for the treatment of diarrhoea. We report a novel use of finely ground clinoptilolite as a potential adjuvant in anticancer therapy. Clinoptilolite treatment of mice and dogs suffering from a variety of tumour types led to improvement in the overall health status, prolongation of life-span, and decrease in tumours size. Local application of clinoptilolite to skin cancers of dogs effectively reduced tumour formation and growth. In addition, toxicology studies on mice and rats demonstrated that the treatment does not have negative effects. In vitro tissue culture studies showed that finely ground clinoptilolite inhibits protein kinase B (c-Akt), induces expression of p21WAF1/CIP1 and p27KIP1 tumour suppressor proteins, and blocks cell growth in several cancer cell lines. These data indicate that clinoptilolite treatment might affect cancer growth by attenuating survival signals and inducing tumour suppressor genes in treated cells.

17 A long-term study of feeding a diet supplemented with clinoptilolite to dairy cows showed no adverse effects from week 4 until the end of lactation and the tests conclusion.

[40] Abstract

The objective of the experiment was to investigate the effect of clinoptilolite (a natural zeolite) supplementation in the ration of dairy cows on serum copper (Cu), zinc (Zn), and iron (Fe) concentrations. Fifty-two clinically healthy Holstein cows were randomly assigned to one of three groups according to their age and parity. The first group (group A) comprised 17 cows fed a ration supplemented with 1.25% clinoptilolite, the second group (group B) comprised also 17 cows was given a ration with 2.5% clinoptilolite, and the third group (group C, the control), comprised 18 cows fed the basal ration that did not contain any clinoptilolite. The experiment started when the cows entered the fourth week before the expected parturition and lasted until the end of lactation. All cows were fed the above concentrates during the entire experimental period. Blood samples were collected from each animal at the starting day of the experiment, at the day of calving, and at monthly intervals thereafter. All samples were tested for serum Cu, Zn, and Fe concentrations. The results showed that the 1.25 and 2.5% supplementation of clinoptilolite did not have any adverse effects on serum concentrations of Cu, Zn, and Fe

18 Effects of long-term feeding of a diet supplemented with clinoptilolite to dairy cows on the incidence of ketosis, milk yield and liver function.

[37] Abstract

Fifty-two clinically healthy Holstein cows were randomly assigned to one of three groups according to their age and parity. The first group (A) consisted of 17 cows that were fed a concentrate ration supplemented with 1.25 per cent clinoptilolite, the second group (B) consisted of 17 cows fed a ration supplemented with 2.5 per cent clinoptilolite, and the third group (C) consisted of 18 cows, which were fed the basal ration containing no clinoptilolite. The rations were fed from four weeks before the cows' expected parturition dates until the beginning of the next dry period. Blood samples were collected from each animal at the start of the experiment, on the day of calving and then monthly, and analysed for serum glucose, ketone bodies, liver enzymes, blood urea nitrogen (BUN) and total proteins. The milk yield of each cow was recorded monthly. The cows in group B had significantly fewer cases of clinical ketosis during the first month after calving and a higher total milk yield. Feeding the cows with clinoptilolite for a long period had no apparent adverse effects on their liver function, and did not significantly affect the concentrations of glucose, ketone bodies, BUN and total proteins in their serum.

19 This study showed that forty Japanese quail chicks given clinoptilolite in their food reduced the detrimental effects of aflatoxicosis. At the end of the study there were no mortalities in any of the study groups.

[20] Abstract

Clinoptilolite (CLI, a natural zeolite), incorporated into the diet at 50 g/kg, was evaluated for its ability to reduce the deleterious effects of 2.0 mg total aflatoxin (AF;83.06% AFB1, 12.98% AFB2, 2.84% AFG1 and 1.12% AFG2)/kg diet on growing Japanese quail chicks from 10 to 45 d of age. A total of 40 Japanese quail chicks were divided into 4 treatment groups (control, AF, CLI, AF plus CLI) each consisting of 10 chicks. The performance of the birds was evaluated. The AF treatment significantly decreased food consumption and body weight gain from the 3rd week onwards. The adverse effect of AF on food conversion ratio was also significant from week 4 of the experiment. The addition of CLI to an AF-containing diet significantly reduced the deleterious effects of AF on food consumption, body weight gain and food conversion ratio. Food consumption was reduced by 14% in quail chicks consuming the AF diet without CLI, but by only 6% for quail chicks consuming the AF plus CLI diet. Similarly, overall body weight gain was reduced by 27% in birds consuming the AF diet without CLI, but by only 8% for birds consuming the AF plus CLI diet. The addition of CLI to the AF-free diet significantly decreased food consumption and body weight gain during

week 4, but these parameters were similar to the controls in week 5. No mortality was observed in any of the groups. These results suggest that CLI effectively diminished the detrimental effects of AF on the variables investigated in this study.

20 In a trial of rats fed a contaminated diet containing aflatoxin and sorbent materials (natural zeolites) it was determined that the sorbents by themselves had no toxic effects.

[12] Abstract

Hydrated sodium calcium aluminosilicate (HSCAS), a sorbent compound obtained from natural zeolite, has demonstrated an ability to sorb aflatoxins (AFs) with a high affinity. Addition of this compound to feedstuffs contaminated with AFs has shown a protective effect against the development of aflatoxicosis in farm animals. The objective of the present study was to compare the efficiency of HSCAS and local montmorillonite silicate in respect of the protection against aflatoxicosis in the rat as a sensitive animal model. AF treatment (2.5 mg kg^{-1} diet) significantly reduced blood hemoglobin, erythrocytes, leukocytes, cholesterol, triglycerides, cholinesterase, total protein, albumin, zinc and copper concentrations. While it significantly increased creatinine, bilirubin, urea nitrogen, alkaline phosphatase and transaminases concentrations. In addition, AF administration induced degenerative changes in the hepatic and renal tissues. The results indicated that addition of HSCAS or montmorillonite to the AF-contaminated diet at a level of 5 g kg^{-1} resulted in a significant improvement in the hematological and biochemical parameters, mineral retention and histological picture of both liver and kidneys. It is concluded that the deleterious effects of AF could be overcome or, at least, diminished by sorbents. Moreover, sorbents by themselves had no toxic effects

21 Effects of zeolites on cultures of marine micro-algae: The study showed that the zeolite stimulated the growth of phyto-planktons, the basis of all marine food webs, and was non-toxic to these tiny organisms. In fact it made economic sense to include zeolite in fish foods.

[192] Abstract

GOAL, SCOPE AND BACKGROUND: The cation-exchange capacity of zeolites is well known and has been increasingly explored in different fields with both economic and environmental successes. In aquatic medium with low salinity, zeolites have found multiple applications. However, a review of the literature on the applications of zeolites in salt waters found relatively few articles, including some recently published papers. The purpose of this review is to present the state-of-the-art on applications of using zeolites for amending the trace elemental contents of salt water as well as the implications of this property for promoting marine micro-algal growth. MAIN FEATURES: This paper deals with the following features: Sorption capacity of zeolites including 1. application of zeolites in saltwater, 2. the role of silicon and zeolites on cultures of micro-algae, and 3. the role of organically chelated trace metals. RESULTS: The following competing factors have been identified as effects of zeolites on algal growth in salt water: (i) ammonia decrease: growth inhibition reduced; (ii) macro-nutrients increase, mainly silicon: stimulation of silicon-dependent algae; (iii) trace metals increase (desorption from zeolites) or decrease (adsorption): inhibition or stimulation, depending on the nature of the element and its concentration; and, (iv) changes in the chelating organics exudation: inhibition or stimulation of growth, depending on the (a) nature of the complexed element; (b) bioavailability of the complex; and (c) concentration of the elements simultaneously present in inorganic forms. DISCUSSION: Zeolites have been capable of stimulating the growth of the silicon-demanding marine micro-algae, like diatoms, mainly because they can act as a silicon buffer in seawater. Zeolites can also influence the yield of non-silicon-demanding algae, because the changes they can cause (liberation and adsorption of trace elements) in the composition of the medium. CONCLUSIONS: Zeolites have been capable of stimulating the growth of the marine micro-algae. However, the extent of ion exchange between zeolite and seawater, which conditions the effects, will depend on several factors: (1) initial metal concentration in seawater; (2) levels of trace metals in the zeolites (contaminants); (3) characteristics of the zeolites in terms of both ion-exchange capacity and specific affinities for the different cations; (4) quantity of zeolite per litre of solution; (5) pH and (6) response of the organism in terms of liberation of organic ligands. RECOMMENDATIONS AND PERSPECTIVES: RECOMMENDATIONS: Therefore, a previous investigation in each particular case is recommended, in order to select the zeolitic characteristics and concentrations that will maximize the algal yield. PERSPECTIVES: Stimulation of phytoplankton growth can be economically relevant since phytoplankton constitutes the basis of the marine food webs and is required in fish farming nurseries in the marine aquaculture industry. Zeolites are cheap, only small amounts (few milligrams per liter of culture) are required and the addition of some micro-nutrients may be omitted. Therefore, the inclusion of zeolites in algal cultures in aquaculture may have economic advantages

22 The effect of clinoptilolite zeolite on the removal of lead toxicity from fungi without being toxic to the fungi itself.

[110] Abstract

In order to determine whether clinoptilolite, a naturally occurring zeolite, had any ameliorative effect on lead (Pb) toxicity to fungi, a series of growth experiments were performed. Three fungi, Aspergillus niger, Botrytis cinerea, and Fusarium culmorum, were grown on appropriately amended solid agar media, and their linear extension rates determined. B. cinerea was 25% inhibited, as compared to a control, at 100 mg dm(-3) Pb, and completely inhibited at 1000 mg dm(-3) Pb. F. culmorum was completely inhibited, and A. niger 97% inhibited at 1000 mg dm(-3) Pb. The addition of 3% clinoptilolite partially removed this inhibition in the case of A. niger and B. cinerea and almost completely removed it for F. culmorum. At a constant 500 mg dm(-3) Pb, increasing concentrations of clinoptilolite increased the linear extension rate of F. culmorum and B. cinerea, close to the rates achieved by the untreated controls. A. niger was not inhibited markedly at this Pb concentration. The evidence suggests that the Pb is adsorbed by the clinoptilolite which reduces the availability, and hence toxicity, of the metal to the fungi

23 A study in Sweden using de-aluminated zeolite as a method to study endocytosis inside viable human peripheral dendritic cells provided an efficient carrier of bi-molecules into endosomal pathways of viable cells without affecting the cells themselves.

[68] Abstract

We report the use of nanometre-sized zeolite particles as a novel approach to follow the endosomal acidification and proteolysis inside a viable cell. The method was verified by using human peripheral monocytes, a well known endocytosing cell population. Zeolite particles were subsequently used to investigate the endocytosing mechanisms of human peripheral dendritic cells (DC). Probes detecting pH neutral and acidic endosomes were adsorbed to de-aluminated zeolite Y, and used to detect endocytosis in immature human peripheral blood DC. Both the myeloid (mDC) and the plasmacytoid (pDC) dendritic cell subsets had an endocytosing capacity comparable with peripheral blood monocytes. However, the majority of both subsets of DC retained their endosomes at a neutral pH during the first hours after endocytosis and only a small number of the mDC showed any formation of acidic endosomes. Proteolytic degradation of endocytosed proteins was detected using self-quenched DQ-ovalbumin adsorbed to zeolite particles. Interestingly, a clear difference in proteolytic degradation of endocytosed ovalbumin was observed between the two subsets of DC. The mDC showed an efficient degradation of ovalbumin, while the pDC population displayed no or only minor proteolytic degradation. In conclusion, zeolite particles provide a useful tool to study the endocytosing mechanisms, and an efficient carrier of bio-molecules into the endosomal pathways of viable cells

24 The effect of zeolite (clinoptilolite) on the post-feeding dynamics of N metabolism in the portal veins, jugular veins and the rumen fluid in bulls. The zeolite did not affect urea concentration in their blood.

[248] Abstract

If easily digestible saccharides are deficient in the feed ration of bulls with the live weight of 300 kg and at simultaneous single application of urea at a rate of 0.2 g per 1 kg live weight, zeolite (with 50.6% clinoptilolite content) administered at a rate of 2.5% per 1 kg dry matter influenced significantly (P less than 0.05) the ammonia concentration in rumen, v. portae and v. jugularis. The rumen contents and blood were sampled at the intervals of 0, 15, 30, 60, 90, 120, 180 and 360 minutes after feeding. Basal feed ration consisted of 1 kg feed mixture and 3 kg meadow hay. After urea administration, zeolite reduced the ammonia concentration in rumen by 20-40% in comparison with the control group and in v. portae by 60-70%. In v. jugularis in the 90th minute after feeding significant hyperammonemia was observed in bulls with no zeolite supplement. Zeolite administration did not influence urea concentration in plasma.

25 There were no adverse effects of the short-term supplementation of clinoptilolite in colostrum and milk on the concentration of some serum minerals in neonatal dairy calves.

[43] Abstract

In recent years, the use of both natural and synthetic zeolites in animal nutrition has increased mainly to improve their performance, health, and to protect against mycotoxin intoxication. Thirty calves were used in the present study for the determination of some physiologic effects of clinoptilolite supplementation. The animals were divided equally into three groups (control, test 1, and test 2). The three groups of calves were homogeneous for parity of dams, sex, and month of birth. For group test 1, clinoptilolite in the concentration of 2% of each colostrum meal was added for 48 h, and for group test 2, clinoptilolite in the concentration of 2% was added to each colostrum and milk meal for 14 days. Blood samples were taken from all calves 12 h after birth and at the end of the first, second, third, forth, fifth, and sixth weeks of life. Calcium (Ca), phosphorus (P), magnesium (Mg), iron (Fe), sodium (Na), and potassium (K) were determined in the serum. For statistical analysis of data, a repeated measures approach using analysis of variance (ANOVA) with mixed linear models was used. Clinoptilolite supplementation had significant effect on the concentrations of calcium, phosphorus, sodium, and iron. The concentrations of Fe significantly higher in test group 2 than other trial groups ($p < 0.05$). Calcium concentrations were significantly higher in serum of clinoptilolite-treated than control calves ($p < 0.05$). The concentrations of phosphorus were significantly lower in test groups than control group ($p < 0.05$). Sodium concentrations were significantly higher in clinoptilolite-supplemented groups than control calves ($p < 0.05$). Potassium and magnesium concentrations were not affected by clinoptilolite supplementation. Clinoptilolite supplementation could promote iron levels in serum and better hemopoiesis and prevent pathologic or physiologic drop of red blood cell (RBC) parameters in supplemented calves during a first few weeks of life. According to higher need and utilization of Ca in growing animals, clinoptilolite supplementation could increase available Ca. Based on the results of the present study and the importance of dietary phosphorus in many physiologic processes, the level of phosphorus in diet of neonatal dairy calves must be considered and adapted when clinoptilolite was supplemented. With an adequate supply of good quality drinking water, cattle can tolerate large quantities of dietary sodium chloride. Thus, it seems that significant increase in serum Na concentration during short-term supplementation of clinoptilolite in neonatal calves could be well tolerated without any adverse effects.

26 This paper is a review of the role of natural and synthetic zeolites as feed additives in the prevention and/or the treatment of certain farm animal diseases. It comments that zeolites perform many beneficial mechanisms and new research suggests that zeolites have the potential to deliver positive effects in cases of gastro-intestinal occurrences and intestinal parasite infections. There appears to be no safety or toxicity issues.

[49] Abstract

The present review comments on the role of the use of zeolites as feed additives on the prevention and/or the treatment of certain farm animal diseases. Both natural and synthetic zeolites have been used in animal nutrition mainly to improve performance traits and, based on their fundamental physicochemical properties, they were also tested and found to be efficacious in the prevention of ammonia and heavy metal toxicities, poisonings as well as radioactive elements uptake and metabolic skeletal defects. During the last decade, their utilization as mycotoxin-binding adsorbents has been a topic of considerable interest and many published research data indicate their potential efficacy against different types of mycotoxins either as a primary material or after specific modifications related to their surface properties. Ingested zeolites are involved in many biochemical processes through ion exchange, adsorption and catalysis. Recent findings support their role in the

prevention of certain metabolic diseases in dairy cows, as well as their shifting effect on nitrogen excretion from urine to faeces in monogastric animals, which results in lower aerial ammonia concentration in the confinement facilities. Moreover, new evidence provide insights into potential mechanisms involved in zeolites supporting effect on animals suffered from gastrointestinal disturbances, including intestinal parasite infections. All the proposed mechanisms of zeolites' effects are summarized in the present review and possible focus topics for further research in selected areas are suggested.

27 This South African study shows the effect of feeding clinoptilolite (zeolite) on the performance of three strains of laying hens. Although there were differences between the strains of the hens there were no zeolite interactions.

[15] Abstract

One hundred and twenty 16-week-old single combed pullets of three strains were fed on a diet containing 135 g protein/kg with or without 50 g clinoptilolite/kg in a trial with 20 hens per treatment. Sterile river sand replaced clinoptilolite in the control diet in order to keep the diets isoenergetic. The hens were individually caged in a naturally ventilated laying house and fed one of the two diets for ten 28-d periods. 2. Significant dietary effects of feeding clinoptilolite were observed with number of eggs laid per hen, shell thickness, efficiency of food utilisation and droppings moisture content. No significant dietary effects between treatments were observed with body weight, age at first egg, egg weight. Haugh units, food intake/hen and rate of amino acid absorption of radioactive lysine and methionine into the bloodstream. Significant differences between strains were observed with regard to all parameters except food intake/hen. There were no significant strain X clinoptilolite interactions.

28 This study was a toxicological investigation and reviewed all the evidence in relation to the use of Zeolithe 'A' as a phosphate substitute in detergents. It found that, when used in detergents Zeolithe A (a synthetic zeolite) it was not hazardous to human health.

[247] Abstract

Tests on Zeolithe A, a sodium aluminium silicate developed as a substitute for phosphates in detergents, were designed to investigate the safety of exposure to the material, or to detergents containing it, either under industrial conditions encountered during manufacturing processes or as a consequence of domestic use. The test programme included oral studies (acute, subchronic and long-term carcinogenicity tests and absorption measurements), and dermal, ocular and inhalation studies on the silicate alone and on appropriate detergent formulations, as well as studies of possible silicogenic activity and metal-complexing potential and measurements of dust generation and particle-size distribution. These studies did not produce any evidence to suggest that levels of domestic and industrial exposure resulting from the projected use of Zeolithe A in detergents would present any hazard to health. Zeolithe A did not induce silicotic tissue reactions and when incorporated into detergent formulations did not increase the liberation of fine dusts.

29 Pigs feed clinoptilolite NaA were protected against cadmium-induced anemia and their liver minerals were unaffected.

[27] Abstract

Weanling Landrace X Yorkshire swine were fed a basal diet or a diet containing 3% clinoptilolite (a natural zeolite) with or without 150 ppm CdCl2 or 3% zeolite NaA (a synthetic zeolite) with or without 150 ppm CdCl2 for 31 days. Hematocrit and hemoglobin were depressed significantly in animals fed Cd in the absence of zeolites, but not in their presence. Liver Cd concentration was increased dramatically by added dietary Cd but was significantly lower in animals fed clinoptilolite with Cd than in those fed Cd alone (11.4 vs 16.5 ppm). Liver Fe and Zn were decreased by dietary Cd; liver Fe was not affected significantly by clinoptilolite or zeolite NaA, but liver Zn was increased by zeolite NaA. Kidney dry matter, Zn, and Cd concentrations were increased by dietary Cd; neither clinoptilolite nor zeolite NaA affected kidney Cd concentration. Zeolite NaA increased kidney dry matter both in the presence and in the absence of dietary Cd. Plasma urea-N, K, Na, and Mg were unaffected by Cd or by either zeolite. The data illustrate the different effects of dietary clinoptilolite compared with zeolite NaA on blood plasma, liver, and kidney concentrations of minerals and provide evidence that both zeolites offer some protection against Cd-induced Fe-deficiency anemia; the magnitude of this protection and the effects of each zeolite on tissue concentrations of Cd and other materials need further quantification.

30 A case series describing thermal injury resulting from zeolite use for haemorrhage control in combat operations.

[60] Abstract

Four cases are presented to illustrate cutaneous burns sustained with the use of zeolite in the treatment of major hemorrhage secondary to combat wounds. Zeolite, a microporous crystalline aluminosilicate granular hemostatic agent, can cause secondary thermal injuries through an exothermic reaction that is likely related to the absorption of free fluid at the hemorrhage site. Understanding of this process may help both military and civilian EMS personnel avoid or minimize secondary thermal injury while still benefiting from zeolite's hemostatic capabilities

31 What a physician should know about zeolites. This article shows that any free aluminium or silicon is reabsorbed by the zeolite in the intestinal tract before it leaves the body.

[249] Abstract

Zeolites are natural and synthetic hydrated crystalline aluminosilicates endowed with absorptive and ion exchange properties. They have found numerous and multifarous applications--in industry as catalysts and absorbents, in water sanitation for the removal of ammonia and heavy metals, in agriculture as fertilizers, and in animal husbandry as the absorbents of excreted material and as food additives. Medical applications have included the use in filtration systems for anesthesia or dialysis and as the contrast materials in NMR imaging. Recently, zeolite powders for external use have found application as deodorants, antimycotic agents and wound dressings. Peroral use of encapsulated

zeolite powders enriched with vitamins, oligoelements or other ingredients has been claimed to exert beneficial medical effects. Ingestion of zeolites may be considered analogous to the clay eating (geophagia), considered in traditional medicine as a remedy for various illnesses. Being amphoteric, zeolites are partly soluble in acid or alkaline media, but within the physiological pH range the solubility is generally low. Minimal amounts of free aluminium or silicium from the ingested zeolites are reabsorbed from the gut. The bulk of ingested zeolite probably remains undissolved in the gut. In view of the ion exchange properties, zeolites may be expected to change the ionic content, pH and buffering capacity of the gastrointestinal secretions and to affect the transport through the intestinal epithelium. In addition, zeolites could affect the bacterial flora and the resorption of bacterial products, vitamins and oligoelements. The contact of zeolite particles with gastrointestinal mucosa may elicit the secretion of cytokines with local and systemic actions. Reactive silicium ions might react with biomolecules of the intestinal epithelium, and if resorbed, do so in other cells. Mutagenic and carcinogenic effects of zeolite particles have been described, resembling such effects of asbestos fibers. Thus, local and systemic effects of zeolites may be complex and interrelated, and an objective assessment requires appropriate experimental models.

33 QuikClot is a wound dressing approved and deployed by the United States Military to stop bleeding from severe combat wounds.

[61] Abstract

BACKGROUND: QuikClot is a zeolite-based dressing approved and deployed by military for the arrest of severe combat-induced hemorrhage. A novel formulation (bagged QuikClot [ACS]) of the original granular QuikClot (QC) has been proposed to facilitate the application of the hemostatic dressings under battlefield conditions. This study compares the hemostatic efficacy of ACS and QC in controlling blood loss and improving survival in a swine model of uncontrolled hemorrhage induced by complex groin injury. METHODS: After transection of the femoral vasculature, anesthetized Yorkshire pigs (n = 32) were hemorrhaged for 3 minutes and randomized into four groups: no treatment (NONE) or application of standard dressing (SD), QC, or ACS. At 15 minutes, resuscitation was initiated by infusion of 500 mL Hextend during a span of 30 minutes. Vital signs were continuously recorded throughout the 4-hour experimental period. In addition, blood loss and temperature at the dressing and tissue interface were continuously recorded. RESULTS: After 3 minutes, average blood loss was 44.7% +/- 11.9% estimated blood volume (EBV) for all animals (34.1 +/- 3.2 kg). Post treatment blood loss was significantly higher (p < 0.01) for NONE- and SD-treated animals (31.5% +/- 21.8% and 22.3% +/- 12.6% EBV, respectively) as compared with animals treated with QC and ACS (7.4% +/- 7.1% and 10.3% +/- 6.9%, respectively). All NONE animals died at approximately 60 minutes. Times until death were slightly greater for animals treated with SD (96.8 minutes) and significantly greater for animals treated with QC (188 minutes) and ACS (194 minutes). Overall survival to 4 hours for SD (1 of 8, 12.5%) was significantly lower (p < 0.02) than for QC (6 of 8, 75%) and for ACS (6 of 8, 75%) treatments. Elevated temperatures at the dressing and tissue interface were seen in animals treated with QC and ACS (average at 8 minutes was 58.1 +/- 4.5 degrees C and 58.2 +/- 5.3 degrees C, respectively) compared with SD treated animals (38.8 +/- 2.7 degrees C). Histologic examination revealed more edema in muscular tissue of animals treated with ACS as compared with in QC-treated animals. CONCLUSIONS: ACS was as efficacious as original granular QC in inducing hemostasis and improving survival as compared with the efficacy of SD. Easier and more rapid application and complete removal of ACS may offer a distinct advantage in battlefield resuscitation efforts to enhance a clean wound site and eventual surgical repair.

34 Zeolite used in the complex treatment of atonic ulcers with no allergic or toxic reactions.

[254] Abstract

The treatment of the chronic atonic ulcus is a complex process aiming at:

1. A systematic treatment of the basic disease.

2. Cleaning the ulcus by attending local treatment, removing of the superinfection, activation and protection of the granulation and stimulation of the epithelisation.

During the last 2 years, we carried out a clinical experiment which aims to establish the biological action of the mineral substantion Zeolite in the atonic ulcus treatment. According to the preliminary data, the dehydrated Zeolite possesses substantial absorbtion properties, an ability to give iones and stabilise the tissue homeostasis. It was applied to 20 patients with chronic ulcera cruris-5 women and 15 men in advanced age (60-80 years), average age 62 years, with chronic erythemo-erosive and ulcerous changes of the skin. Every case concerned people with damaged peripheral vascular system, a result of postphlebitis states, varices cruris, chronic inflammatory changes following recidivative Erysipellas and Atherosclerosis. After the ulcus sanation with systematic and local antibiotics and removing of the necrotic areas, the preparation has been applied as a 2 mm layer. The bandages have been changed every second day in most of cases, aiming a diminuation of the traumatisation of growing epithelium. Their removing has been accomplished by abundant moisturing with physiological solution and a following washing up through a stream of the same solution under pressure. For every patient, except for one, we observed the same adsorbing effect, the erythema in and around the wounds became paler, the wound surface was drying and transforming in inverse development. The feeling of tension and pain was decreasing progressively. The wounds' reparation, even slow, began from the periphery. None of the patients had a secondary infection, allergic or toxic reaction of intolerance. Clinoptilolite demonstrated excelent antiinflamative, astringent effects and intensificated the epitelisation.

35 Diagnostics, prophylactics and a summary of the healing observations of clinoptilolite.

[252] Abstract

- *Clinoptilolite (a natural zeolite) has no carcinogenic effect on laboratory animals.*
- *Observed a certain tendency toward reduction in tumour formation in mice, especially the Lymphomas – Lymphosarcoma and Reticulum-cell neoplasm type B.*
- *Experimentally inflicted wounds treated with zeolite heal quickly. Locally applied only once to dermal wounds.*
- *Clinoptilolite may be applied and administered for the decrease and relief of acidity and pains in the stomach, as well as similar ailments in the abdominal area, including nutritive, alcoholic, and exo- and endo-geneous intoxications.*
- *Clinoptilolite may also be applied and administered in cases of enhanced cancer danger, or in the cases of natural inclination towards cancerous formations in the human body.*
- *Clinoptilolite may be taken prophylactically – or if required – twice daily, in doses of 2.5 to 5 grams before breakfast and before going to bed in the evening.*

36 Antifungal effect of zeolite-incorporated tissue conditioner against Candida Albicans growth and/or acid production.

[251] Abstract

A new antimicrobial material, Ag-zeolite (Zeomic), was combined with a commercial tissue conditioner (GC-Soft Liner (GC); 1-5%) and, through monitoring the pH of the growth medium, examined for effects on the in vitro growth and/or acid production of Candida albicans on protein-free and saliva-coated specimens. The effect of incorporation of this agent on the physical property of the lining material was also examined according to the ISO penetration test. Comparison studies were carried out using GC, Coe Comfort (CC) or undecylenate combined GC (1-5%) specimens. Although the pH changes in the media varied depending upon the materials on which the Candida was grown, reverse sigmoidal pH curves were observed with most samples. As compared with GC, the soft lining materials showed, to some extent, an inhibitory effect on the acid production and/or the growth of C. albicans. These inhibitory effects consisted of a delay in the onset of rapid pH decline, decreases in the rate of pH change and increases in minimum pH. In most cases, the inhibitory effects of test specimens were dose-dependent, and zeolite specimens showed a significantly higher antifungal effect, followed by CC and undecylenate-combined GC; GC showed the least antifungal effect. The inhibitory effects of these materials on fungal growth were decreased by the presence of a saliva-coat, particularly with zeolite specimens and CC. However, four of eight 5%-Zeomic specimens still exhibite perfect growth inhibition in the presence of the salivary pellicle. Furthermore, test specimens containing 2-5% Zeomic showed a significantly greater effect on the delay in rapid decline of pH, as compared with the other specimens examined. In addition, the significantly higher minimum pH was observed where the yeasts were grown on 4%- and 5%-Zeomic specimens. The physical properties of all the test specimens conformed with the ISO standard as examined by penetration test. These results taken together suggest that an antimicrobial zeolite-combined tissue conditioner would be a potential aid in denture plaque control.

37 Anti-bacterial zeolite balloon catheter to control urinary tract infection showed no adverse affects after insertion for 3 to 7 months.

[250] Abstract

We present here a production of anti-bacterial zeolite balloon catheter and investigated its potential for controlling urinary tract infection. This anti-bacterial balloon catheter showed a bactericidal effect against Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli in vitro studies. The antibacterial effects were correlated with the concentration of anti-bacterial zeolite and size of catheter. We tried this catheter for 11 various urological patients who needed a long-term indwelling of a balloon catheter for lower urinary tract obstruction and neurogenic bladder. All patients were already indwelled silicon balloon catheter for 3 to 6 months and suffered with complicated urinary tract infection. Nine patients who had this anti- bacterial zeolite balloon catheter indwelled for 3 to 7 months and exchanged every 2 to 4 weeks, and no patient was taking antibiotics during this trial. Two patients (22.2%) showed good results by the urinary tract infection (UTI) criteria and 5 patients (55.4%) showed good effects by doctor's judgment. This anti-bacterial zeolite balloon catheter might be useful for patients who need long-term balloon catheter indwelling.

38 Proposal to use zeolites as wound care material for the reduction of noxious odours and pus due to their absorption qualities and promotion of patient health promotion.

Supervisors: Dr Katherine Huddersman huddzeo1@dmu.ac.uk , Dr Andy Barnes abarnes@dmu.ac.uk and Prof Denis Anthony, School of Nursing and Midwifery danthony@dmu.ac.uk

[253] Abstract

Chronic wounds are a major clinical problem; for example pressure ulcers occur in 5-15% of all hospital admissions. Certain types of wounds, particularly pressure ulcers and those due to cancer, develop noxious odours due to tissue necrosis and/or infection. This odour severely damages the patient's quality of life, particularly where a terminal care situation is involved. The odour is due to the production of a range of short-chain organic acids, together with putrescine and cadaverine.

Zeolites act as molecular sieves, whereby organic molecules are selectively absorbed according to size. They also act as catalysts, promoting the oxidation of organic molecules. Zeolites allow chemical destruction of odour-producing molecules, in addition to their sorption, they are potentially superior to traditional products. Zeolites will be investigated for their utility in the absorption and catalytic decomposition of clinically relevant odour molecules and simultaneous removal of exudate.

39 Pharmacokinetic study of zeolite A, sodium aluminosilicate, magnesium silicate, and aluminium hydroxide in dogs.

[51] Abstract

Zeolite A is a synthetic zeolite which may have therapeutic utility in osteoporotic individuals because of its ability to stimulate bone formation. A study of Zeolite A (30 mg/kg), sodium aluminosilicate (16 mg/kg), magnesium trisilicate (20 mg/kg), and aluminum hydroxide (675 mg) was designed in beagle dogs. The purpose of this study was to compare the oral bioavailability of silicon and aluminum from Zeolite A, sodium aluminosilicate, magnesium trisilicate, and aluminum hydroxide in dogs. Twelve female dogs received each compound as a single dose separated by one week in a randomized, 4-way, crossover design. Plasma samples were drawn at time 0 and for 24 hours after dosing. The concentrations of silicon and aluminum were determined by graphite furnace atomic absorption. The mean plasma silicon AUC values (+/- S.D.) were 9.5 +/- 4.5, 7.7 +/- 1.6, 8.8 +/- 3.0, 6.1 +/- 1.9 mg.hr/L and the mean plasma silicon Cmax values (+/- S.D.) were 1.07 +/- 1.06, 0.67 +/- 0.27, 0.75 +/- 0.31, 0.44 +/- 0.17 mg/L for Zeolite A, sodium aluminosilicate, magnesium trisilicate, and aluminum hydroxide respectively. Although mean silicon AUC and Cmax values were elevated when compared to baseline after administration of the silicon containing compounds, only the AUC from Zeolite A reached statistical significance (p = 0.041). The mean plasma silicon Tmax values (+/- S.D.) were 7.9 +/- 6.4, 5.8 +/- 4.6, 6.9 +/- 6.3 and 8.5 +/- 3.4 hrs for Zeolite A, sodium aluminosilicate, magnesium trisilicate and aluminum Hydroxide respectively.(ABSTRACT TRUNCATED AT 250 WORDS)

40 Natural zeolite clinoptilolite: new adjuvant in anticancer therapy.

[58] Abstract

Natural silicate materials, including zeolite clinoptilolite, have been shown to exhibit diverse biological activities and have been used successfully as a vaccine adjuvant and for the treatment of diarrhoea. We report a novel use of finely ground clinoptilolite as a potential adjuvant in anticancer therapy. Clinoptilolite treatment of mice and dogs suffering from a variety of tumour types led to improvement in the overall health status, prolongation of life-span, and decrease in tumours size. Local application of clinoptilolite to skin cancers of dogs effectively reduced tumour formation and growth. In addition, toxicology studies on mice and rats demonstrated that the treatment does not have negative effects. In vitro tissue culture studies showed that finely ground clinoptilolite inhibits protein kinase B (c-Akt), induces expression of p21WAF1/CIP1 and p27KIP1 tumour suppressor proteins, and blocks cell growth in several cancer cell lines. These data indicate that clinoptilolite treatment might affect cancer growth by attenuating survival signals and inducing tumour suppressor genes in treated cells It can be seen from all the above research papers and articles that there is overwhelming amount of evidence that indicates that zeolite, particularly clinoptilolite, is safe to apply to the skin, to put on open wounds and is non-toxic when ingested.

It can be seen from all the above research papers and articles that there is overwhelming amount of evidence that indicates that zeolite, particularly clinoptilolite, it is safe to apply to the skin, to put on open wounds and is completely non-toxic when ingested.

If you have any comments or questions they should be directed to info@natroceuticals.com.