Safety and efficacy of Mintrex®Zn (Zinc chelate of hydroxy analogue of methionine) as feed additive for all species

Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed

(Question No EFSA-Q-2007-098)

Adopted on 16 April 2008

PANEL MEMBERS

Georges Bories, Paul Brantom, Joaquim Brufau de Barberà, Andrew Chesson, Pier Sandro Cocconcelli, Bogdan Debski, Noël Dierick, Anders Franklin, Jürgen Gropp, Ingrid Halle, Christer Hogstrand, Joop de Knecht, Lubomir Leng, Anne-Katrine Lundebye Haldorsen, Alberto Mantovani, Miklós Mézes, Carlo Nebbia, Walter Rambeck, Guido Rychen, Atte von Wright and Pieter Wester

SUMMARY

Following a request from the European Commission, the European Food Safety Authority was asked to deliver a scientific opinion on the safety and efficacy of a zinc chelate of the hydroxy analogue of methionine (Mintrex®Zn) as a feed additive for all species.

Mintrex®Zn contains a minimum of 16 % zinc and 80 % hydroxy methionine analogue ((2-hydroxy-4-methylthio)butanoic acid, HMTBa), as shown from analyses of the product. It is intended to be used as a source of the essential trace element zinc. Zinc (in several forms) and HMTBa are already separately authorised as nutritional feed additives in the European Union.

Mintrex®Zn can be considered as a bioavailable zinc source, comparable to another authorised inorganic source of zinc, for all animal species.

The Panel on Additives and Products or Substances use in Animal Feed (FEEDAP) considers Mintrex®Zn as a new compound of trace elements which therefore requires demonstration of safety for the target species. Since no studies on the tolerance of target animals to Mintrex®Zn were provided, the FEEDAP Panel cannot conclude on the safety of the compound for target animals.

No data on edible tissues and products deposition were provided other than for liver from chickens and milk from dairy cows fed Mintrex®Zn. From the limited available data, the FEEDAP Panel considers it unlikely that the use of Mintrex®Zn would alter zinc deposition in edible tissues/products compared to other zinc sources.

For citation purposes: Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) on a request from the European Commission on the safety and efficacy of Mintrex®Zn (Zinc chelate of hydroxy analogue of methionine) as feed additive for all species. The EFSA Journal (2008) 694, 1-16
Based on the data from acute toxicity and genotoxicity studies, the FEEDAP Panel considers that Mintrex®Zn does not introduce any additional toxicity compared to other sources of dietary zinc.

With regards to consumer safety, the FEEDAP Panel retains zinc as the component of potential toxicological significance of Mintrex®Zn. A model calculation was made based on recent data on zinc intake, and a higher zinc deposition in tissues compared to authorised zinc sources, taken as a worst case scenario. The FEEDAP Panel also recognised that zinc in muscle tissue appears insensitive to zinc supplemented within authorised levels compared to liver and kidney. As a result, consumer exposure would not exceed the upper intake level for zinc (25 mg day\(^{-1}\)). Therefore the FEEDAP Panel concludes that no concerns for consumer safety would result from the use of Mintrex®Zn in feed at the maximum authorised zinc levels.

The FEEDAP Panel considers that Mintrex®Zn is safe for the user provided that protective measures are taken and that it does not represent additional risks to the environment compared to other sources of zinc for which it will substitute.

The FEEDAP Panel made some recommendations with regards to the Register entry and highlighted the need for analytical methods specific to the determination of the chelates rather than the trace element.

**Key words:** nutritional additive, trace element, Mintrex®Zn, zinc, chelate, HMTBa, hydroxy methionine, bioavailability, efficacy, safety, chickens for fattening
TABLE OF CONTENTS

Panel Members ........................................................................................................................................... 1
Summary ...................................................................................................................................................... 1
Table of Contents ....................................................................................................................................... 1
Background ................................................................................................................................................. 4
Terms of reference ....................................................................................................................................... 4
Acknowledgements ...................................................................................................................................... 4
Assessment .................................................................................................................................................. 6
1. Introduction ........................................................................................................................................ 6
2. Characterisation of the product .......................................................................................................... 6
   2.1. Description of the product ........................................................................................................ 6
   2.2. Production .................................................................................................................................. 6
   2.3. Composition ............................................................................................................................... 6
   2.4. Physicochemical properties ....................................................................................................... 6
   2.5. Stability ...................................................................................................................................... 7
   2.6. Homogeneity .............................................................................................................................. 7
   2.7. Conditions of use ....................................................................................................................... 7
   2.8. Evaluation of the analytical methods by the Community Reference Laboratory (CRL) ....... 8
3. Efficacy ............................................................................................................................................... 8
   3.1. Bioavailability ............................................................................................................................ 8
   3.2. Conclusions on efficacy ............................................................................................................. 9
4. Safety for the target species ................................................................................................................ 9
5. Tissue/products deposition ................................................................................................................. 9
   5.1. Conclusions on tissue/products deposition .............................................................................. 10
6. Safety for the consumer ....................................................................................................................... 10
   6.1. Toxicity studies .......................................................................................................................... 10
       6.1.1. Acute toxicity ..................................................................................................................... 10
       6.1.2. Genotoxicity studies including mutagenicity ................................................................... 10
       6.1.3. Conclusions ....................................................................................................................... 11
   6.2. Consumer exposure .................................................................................................................... 11
   6.3. Conclusions on safety for the consumers ................................................................................. 12
7. Safety for the user ............................................................................................................................... 12
8. Safety for the environment .................................................................................................................. 12
9. Post-market monitoring ........................................................................................................................ 12
Conclusions and Recommendations ....................................................................................................... 12
General Remark ......................................................................................................................................... 14
Documentation provided to EFSA ............................................................................................................ 14
References .................................................................................................................................................. 14
Appendix .................................................................................................................................................... 16
BACKGROUND

Regulation (EC) No 1831/2003\(^2\) establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lies down that any person seeking an authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from the company Novus Europe SA,\(^3\) for authorisation of zinc chelate with the hydroxy analogue of methionine (Mintrex®Zn) to be used as a feed additive for all species (category: nutritional additives; functional group: compounds of trace elements) under the conditions mentioned in Table 1.

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4.1 (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application. According to Article 8 of that Regulation, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. The particulars and documents in support of the application were considered valid by EFSA as of 3\(^{rd}\) of September of 2007.

The additive Mintrex®Zn is a zinc chelate of hydroxy analogue of methionine. Nine compounds of zinc are authorised in the EU as sources of trace elements for all animal species without a time limit.\(^4,5\) Among these, two are chelates: the zinc chelate of amino acids (derived from hydrolysed soya protein) hydrate and the zinc chelate of glycine (synthetic) hydrate. The hydroxy analogue of methionine is authorised as nutritional additive in the functional group aminoacids, their salts and derivatives.\(^6\)

The Scientific Committee on Animal Nutrition (SCAN) issued an opinion on the use of zinc in feedingstuffs (14 March 2003).\(^7\) EFSA issued an opinion on the safety of the “Chelated forms of iron, copper, manganese and zinc with synthetic feed grade glycine” (29 November 2005).\(^8\)

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003 EFSA shall determine whether the feed additive complies with the conditions laid down in Article 5. Therefore, EFSA shall deliver an opinion on the efficacy and safety for the target animals, consumer, user and the environment, of zinc chelate of the hydroxy analogue of methionine (Mintrex®Zn), when used under the conditions described in Table 1.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group on Trace Elements for the preparation of this opinion.

---

\(^2\) OJ L 268, 18.10.2003, p.29
\(^3\) Avenue Marcel Thiry 200. B-1200 Brussels
\(^4\) OJ L 187, 26.07.2003, p.11
\(^5\) OJ L 86, 24.03.2006, p.4
\(^7\) http://ec.europa.eu/food/fs/se/scan/out120_en.pdf
\(^8\) http://www.efsa.europa.eu/EFSA/efsalogale-1178620753812_1178620783692.htm
Table 1. **Register entry as proposed by the applicant**

<table>
<thead>
<tr>
<th>Additive</th>
<th>Mintrex® Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Registration number/EC No/No (if appropriate)</td>
<td>3b6.xx</td>
</tr>
<tr>
<td>Category of additive</td>
<td>Nutritional</td>
</tr>
<tr>
<td>Functional group of additive</td>
<td>Compounds of trace elements</td>
</tr>
</tbody>
</table>

### Description

<table>
<thead>
<tr>
<th>Composition, description</th>
<th>Chemical formula</th>
<th>Purity criteria (if appropriate)</th>
<th>Method of analysis (if appropriate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc chelate of hydroxy analogue of methionine 16% Zn 80% HMTBa</td>
<td>Zn-(HMTBa):</td>
<td>Complies with EU law on undesirable substances</td>
<td>Atomic Absorption Spectrometry</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Trade name (if appropriate)</th>
<th>Mintrex® Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name of the holder of authorisation (if appropriate)</td>
<td>Novus® Europe SA</td>
</tr>
</tbody>
</table>

### Conditions of use

<table>
<thead>
<tr>
<th>Species or category of animal</th>
<th>Maximum Age</th>
<th>Maximum content of the element (mg kg⁻¹ of complete feedingstuffs)</th>
<th>Withdrawal period (if appropriate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All species</td>
<td>-</td>
<td>Pet animals: 250 (total) Fish: 200 (total) Milk replacers: 200 (total) Other species: 150 (total)</td>
<td>Not relevant</td>
</tr>
</tbody>
</table>

### Other provisions and additional requirements for the labelling

<table>
<thead>
<tr>
<th>Specific conditions or restrictions for use (if appropriate)</th>
<th>Feed formulations should be adjusted to account for the methionine activity of HMTBa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific conditions or restrictions for handling (if appropriate)</td>
<td>For user safety: breathing protection during handling, safety glasses and gloves. Store in original closed packaging, in cool, dry place.</td>
</tr>
<tr>
<td>Post market monitoring (if appropriate)</td>
<td>Novus Europe SA will conduct post-marketing monitoring in compliance with EU law on feed hygiene, namely by use of HACCP and Traceability systems, and formal monitoring of customer feedback through product or service complaints.</td>
</tr>
<tr>
<td>Specific conditions for use in complementary feedingstuffs (if appropriate)</td>
<td>To supply Zn in final feeds within EU legal limits for each species.</td>
</tr>
</tbody>
</table>

### Maximum Residue Limit (MRL) (if appropriate)

<table>
<thead>
<tr>
<th>Marker residue</th>
<th>Species or category of animal</th>
<th>Target tissue(s) or food products</th>
<th>Maximum content in tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not relevant</td>
<td>Not relevant</td>
<td>Not relevant</td>
<td>Not relevant</td>
</tr>
</tbody>
</table>
1. **Introduction**

Mintrex® Zn is a chelate containing a minimum of 16 % zinc and 80 % hydroxy methionine analogue ((2-hydroxy-4-methylthio)butanoic acid, HMTBa), according to the specifications provided by the applicant. It is intended to be used as a source of the essential trace element zinc for all animal species.

The biological role, requirements/recommendations, deficiency and toxicity symptoms in farm animals have been already described in a former SCAN Opinion (EC, 2003a). To the knowledge of the FEEDAP Panel, there is no additional relevant information that may lead to modify the SCAN Opinion.

2. **Characterisation of the product**

2.1. **Description of the product**

The product is composed of zinc bis(-2-hydroxy-4-methylthio)butanoic acid and a maximum of 1 % mineral oil (specifications not given). The formula is \( \text{Zn(C}_2\text{H}_5\text{S(C}_2\text{H}_4\text{CH(OH)-COO}_2 \) and its molecular weight is 363.8 Daltons. The CAS number of the complexed compound is 292140-29-5. The molecular structure of the zinc-HMTBa chelate has been examined by single crystal X-ray diffraction.\(^9\)

2.2. **Production**

Mintrex® Zn is manufactured by mixing synthetic liquid HMTBa with inorganic zinc oxide under specific heat and moisture conditions in a reactor/dryer.\(^10\) The dry Mintrex® Zn chelate is milled to a standard particle size and then blended with feed grade mineral oil.

2.3. **Composition**

Based on the analyses of ten batches, the additive contents of HMTBa and zinc were 81.1±0.5 % and 17.5±0.4 %, respectively. Mintrex® Zn is declared to contain not more than 1 % of mineral oil (not confirmed by analytical data).

In three different batches, microbial impurities, mycotoxins (aflatoxin B1), heavy metals and dioxins were determined. Microbial contamination and aflatoxin content (<0.001 mg kg\(^{-1}\) Mintrex® Zn) did not raise concern. Contents of As, Cd, Pb, Hg and F were found to be <0.5, <0.8, <3.2, <0.1 and <0.15 mg kg\(^{-1}\) Mintrex® Zn, respectively. Dioxins were found at levels of 0.102 to 0.107 ng WHO TEQ kg\(^{-1}\) Mintrex® Zn. Dioxin like PCBs were not analysed.\(^11\)

2.4. **Physicochemical properties**

The final product is a light grey powder composed of spherical granules with a bulk density of 1.04 g (cm\(^3\))\(^{-1}\). Around 10 % of particles showed a diameter of <105 µm; this fraction is considered inhalable. The respirable fraction (particles ≤ 10µm) was not determined.

---

\(^9\) Technical Dossier, Section II, Annex II.2.2.4.2.  
\(^10\) Technical Dossier, Section II  
\(^11\) Technical Dossier, Section II
Dusting potential was examined in one batch only and the product classified as ‘fairly dusty’ by the applicant (based on dustiness measured by ‘Dustmon L Anatec’ apparatus).\textsuperscript{12}

2.5. Stability

A method for direct determination of the zinc complex in Mintrex\textsuperscript{®}Zn was not provided. The stability of the chelate has therefore not been directly analysed. Instead, stability was derived from the calculation of changes in the free HMTBa, assuming that the zinc/HMTBa ratio in Mintrex\textsuperscript{®}Zn would remain constant and that HMTBa does not interact with any other components.\textsuperscript{13}

The applicant proposes a provisional expected shelf life of three years. At present, the shelf life of Mintrex\textsuperscript{®}Zn has only been demonstrated for 12 months under standard conditions (25ºC, 60 % RH) and for six months under accelerated conditions (40ºC, 75 % RH). Calculated recoveries of complexed zinc from three batches at the end of the test periods showed values of 103 % and 101 % for standard and accelerated conditions, respectively; the respective recovery rates of total HMTBa were 101 and 100 %. Recovery rates of total HMTBa were 100 %. Stability towards light, air oxygen and pH of Mintrex\textsuperscript{®}Zn is considered by the applicant to be guaranteed by packaging the additive in light-tight sealed bags when stored under normal atmospheric conditions.

All data on HMTBa stability in feed and premixtures are mean values obtained from stability studies performed with Mintrex\textsuperscript{®}Cu, Mintrex\textsuperscript{®}Zn and Mintrex\textsuperscript{®}Mn given together.

In premixtures, mash feed, pelleted feed and during pelleting (in each case at both standard and accelerated conditions), zinc concentration, as expected, remained constant. HMTBa measurements could not be attributed to any one of the Mintrex\textsuperscript{®} products.

2.6. Homogeneity

Homogeneity data were based on ten samples each of premixtures, pelleted and mash feed collected from one lot of Mintrex\textsuperscript{®}Zn. The coefficient of variation (CV) of zinc from Mintrex\textsuperscript{®}Zn was 1.3 % in premixtures, 15.6 % in mash feed and 11.8 % in pellets.

The data indicate that Mintrex\textsuperscript{®}Zn can be homogeneously distributed in premixtures and, to a lesser extent, in mash and pelleted complete feedingstuffs.\textsuperscript{14}

2.7. Conditions of use

Mintrex\textsuperscript{®}Zn is intended to supply zinc in final feed within EU legal limits for all species (150 mg kg\textsuperscript{-1} complete feed, except for the following: milk replacers, 200 mg kg\textsuperscript{-1} complete feed; fish, 200 mg kg\textsuperscript{-1} complete feed; pet animals, 250 mg kg\textsuperscript{-1} complete feed).

According to the applicant, feed formulations should be adjusted to account for the methionine efficacy of HMTBa in Mintrex\textsuperscript{®}Zn.

According to the current knowledge, no incompatibilities or adverse interactions –with feed components, carriers, other approved additives or medical products– are to be expected other than those widely recognised for zinc in animal nutrition.

\textsuperscript{12} Technical Dossier, Section II
\textsuperscript{13} Technical Dossier, Section II. Supplementary information. August 2007
\textsuperscript{14} Annex II.2.3.1.
2.8. Evaluation of the analytical methods by the Community Reference Laboratory (CRL)

EFSA has verified the report submitted by the Community Reference Laboratory (CRL) concerning the analytical method(s) for Mintrex®Zn. The executive summary of the report can be found in the Appendix.

3. Efficacy

Evidence of in vivo bioavailability is required to support efficacy for compounds of trace elements not already authorised as feed additives. A single trial in a single animal species/category, including laboratory animals, is considered sufficient.

Bone and pancreatic zinc are the best response criteria to assess the biological value of zinc sources in monogastrics, while in ruminants liver and kidney accumulation are more appropriate parameters (Jongbloed et al., 2002).

Studies with Mintrex® in which the diet was supplemented simultaneously with Mintrex®Cu, Mintrex®Mn and Mintrex®Zn, are not considered further in the present assessment because potential interactions could not be excluded.

3.1. Bioavailability

Four hundred and thirty-two day-of-hatch male chickens for fattening (Ross x Ross 308) were allocated to nine dietary treatments. Each treatment was replicated eight times with six male chickens per pen. A semi-purified basal diet was designed to meet or exceed all dietary recommendations for chickens for fattening from 1 to 21 days of age, except for zinc (8.5 mg kg⁻¹ analysed value). To deplete the zinc status of all chickens, the zinc-deficient basal diet was fed from day 0 to day 7. The chickens were then fasted for 16 hours between day 7 and day 8. From day 8 to day 21, the birds in the experimental groups were fed the basal diet supplemented with zinc at levels of 6.5, 23.5, 38.5 and 83.5 mg kg⁻¹ from either Mintrex®Zn or zinc sulphate (ZnSO₄·7H₂O). Except for the 16-hour fast, water and feed were given ad libitum from day 0 to 21. On day 21, body weight and feed intake were recorded for each pen. The left tibia and right lobe of the liver were sampled from one chicken per pen for a zinc analysis. The foot pad and a slice of the proximal right tibia were collected for histological examination.

Table 2. Effect of source and level of supplemental zinc on performance and zinc concentrations in liver and tibia of chickens for fattening aged 21 days

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Supplemental level of zinc mg kg⁻¹</th>
<th>Mintrex®Zn</th>
<th>Zinc sulphate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>6.5</td>
<td>23.5</td>
</tr>
<tr>
<td>Feed intake, g bird⁻¹</td>
<td>247bcd</td>
<td>347b</td>
<td>523a</td>
</tr>
<tr>
<td>Body weight gain, g bird⁻¹</td>
<td>67c</td>
<td>198d</td>
<td>376bc</td>
</tr>
<tr>
<td>Gain/Feed, g g⁻¹</td>
<td>0.28d</td>
<td>0.57c</td>
<td>0.72ab</td>
</tr>
<tr>
<td>Liver zinc, mg kg⁻¹</td>
<td>84.8bcd</td>
<td>82.8bcd</td>
<td>80.2cd</td>
</tr>
<tr>
<td>Tibia zinc, mg kg⁻¹</td>
<td>34.9d</td>
<td>40.5d</td>
<td>73.0c</td>
</tr>
</tbody>
</table>

a,b,c,d: means with different superscripts within a row differ significantly (P<0.05)

Regardless of source, performance with respect to body weight gain, feed intake and gain to feed ratio was positively affected by the increase in supplemental zinc (Table 2). The increase in each of the parameters was curvilinear, where breakpoint analysis indicated a required

15 Technical Dossier, Section III, Annex III.3.2.Zn2
minimum zinc supplementation of 25 mg kg\(^{-1}\) for body weight gain and 33 mg kg\(^{-1}\) for gain/feed. There was no significant difference in the efficacy between Mintrex\textsuperscript{®} Zn and zinc sulphate for the performance parameters, which supports the bioequivalence of zinc from Mintrex\textsuperscript{®} Zn and from zinc sulphate.

Tibia zinc deposition resulted in a significant 3.5-fold increase in the group supplemented with the highest zinc dose (83.5 mg kg\(^{-1}\)), regardless the source, compared to negative control. Histological analyses showed that, regardless the source, bone structure was more developed as the level of zinc supplementation increased.

### 3.2. Conclusions on efficacy

Mintrex\textsuperscript{®} Zn shows the same pattern as zinc sulphate in increasing tibia bone zinc in chickens for fattening when doses between 6.5 and 83.5 mg zinc kg\(^{-1}\) from either source were added to a zinc deficient basal diet. Since inorganic zinc sulphate is generally recognised and used as a source of available zinc in animal nutrition, Mintrex\textsuperscript{®} Zn can be considered as a bioavailable zinc source, comparable to zinc sulphate, for all animal species.

### 4. Safety for the target species

Zinc is recognised to possess a low to moderate oral toxicity in farm animals. With a well-balanced diet, maximum tolerable levels of zinc of 300 and 500 mg kg\(^{-1}\) for sheep and cattle, respectively, and 1000 mg kg\(^{-1}\) for swine, chickens and turkeys, were set by the National Research Council (NRC, 1980).

The FEEDAP Panel considers that tolerance of the target animals should be demonstrated for compounds of trace elements not previously authorised. When the application concerns all animal species, one tolerance study with the most sensitive species (or even a laboratory animal) may be sufficient.

As for efficacy assessment, studies with Mintrex\textsuperscript{®} in which the diet contains the three trace elements copper, manganese and zinc from Mintrex\textsuperscript{®} given simultaneously are not further considered because potential interactions cannot be excluded.

Due to the lack of tolerance tests performed with Mintrex\textsuperscript{®} Zn, the FEEDAP Panel cannot conclude on the safety of Mintrex\textsuperscript{®} Zn for target species.

The HMTBa contribution from Mintrex\textsuperscript{®} Zn needs consideration to keep total dietary methionine at desired levels.

### 5. Tissue/products deposition

Within the range of homeostatic regulation, tissue storage of zinc increases only slightly as dietary zinc increases, this not being the case in the presence of excessive amounts of the metal (NRC, 1980). In general, the zinc content of liver, kidney and muscles remains low when zinc is used about requirement levels; however, with additional zinc intake within authorised levels, zinc content in liver and kidney is increased while muscle zinc remains essentially unchanged (Jenkins and Hidiroglou, 1991). No differences in liver, kidney and pancreas zinc deposition were observed in lambs with excessive intake of zinc between inorganic and organic (including chelated) sources of zinc (Cao et al., 2000). In a recent study, there were no differences in eggs zinc deposition when zinc from inorganic or organic (excluding Mintrex\textsuperscript{®}) sources was supplied to feed for laying hens within EU authorised levels (Huyghebaert et al., 2006).
In the study already described (see Section 3.1), the use of Mintrex®Zn and zinc sulphate resulted in comparable liver concentrations. No further data on zinc deposition in edible tissue were provided from experiments using Mintrex®Zn alone.

In a study on dairy cows, the effects of simultaneous feed supplementations with Mintrex® chelates of zinc, manganese and copper were compared to equivalent doses of zinc oxide. The experiment lasted 103 days and showed no significant differences in milk and hair zinc concentrations between the negative control, the Mintrex®Zn group and the group supplemented with the inorganic source of zinc. However, the total zinc levels in feeds of supplemented groups reached only the 69 % value of the maximum EU authorised level.

5.1. Conclusions on tissue/products deposition

Measured values for zinc in chicken liver responded similarly to the level of supplementation for both Mintrex®-Zn and zinc sulphate. Dietary supplementation with zinc had no effect on zinc excretion via milk. No other data on tissue deposition were provided. However, the FEEDAP Panel recognises that, from other published data, zinc in muscle tissue appears insensitive to zinc supplementation, within authorised levels, compared to liver and kidney.

From the limited experimental data available, the FEEDAP Panel considers it unlikely that the use of Mintrex®Zn would alter zinc deposition in edible tissues/products compared to other zinc sources.

6. Safety for the consumer

6.1. Toxicity studies

The applicant presented acute oral toxicity and genotoxicity studies on Mintrex®Zn.

6.1.1. Acute toxicity

In an acute toxic class dose study (OECD, 2001) female rats were treated by oral gavage with 300 or 2000 mg Mintrex®Zn kg⁻¹ bw. Two out of three rats died within day 2 following treatment at the top dose. Signs of toxicity at 300 mg kg⁻¹ bw were limited to post-dosing salivation. Accordingly, the median lethal dose was estimated to be between 500 and 1000 mg Mintrex®Zn kg⁻¹ bw.

6.1.2. Genotoxicity studies including mutagenicity

Mutagenicity of Mintrex®Zn was assessed in a reverse mutation assay on five S. tiphymurium strains (TA98, TA100, TA1535, TA1537 and TA102) with and without metabolic activation. No significant increase of revertant colonies was observed up to the limit concentration of 5000 µg mL⁻¹.

Mintrex®Zn was also tested in CHO cells for chromosomal aberrations with and without metabolic activation. A significant increase of structural chromosomal aberrations was seen only at cytotoxic concentrations of 250 and 400 mg L⁻¹, without and with S-9 activation, respectively. At such concentrations, cytotoxicity was in the 50 % range.
Genotoxicity was assessed *in vivo* in an oral bone marrow micronucleus assay on adult mice which were administered the feed additive once daily at various concentrations for two consecutive days. No genotoxic effects were observed following the second administration with 125, 250 or 500 (maximum tolerated dose) mg Mintrex®Zn kg-1 bw day-1 in female mice or up to 1000 mg kg-1 bw day-1 in male mice.

### 6.1.3. Conclusions

Mintrex®Zn shows low acute toxicity in laboratory rodents and is not mutagenic *in vitro*. It increases chromosomal aberrations *in vitro* but at cytotoxic concentrations and it was not genotoxic in a micronucleus mouse assay. Therefore, the FEEDAP Panel considers that Mintrex®Zn does not introduce any additional toxicity effects compared to other sources of dietary zinc.

### 6.2. Consumer exposure

Although no data was supplied on the metabolic fate of zinc-HMTBa, the FEEDAP Panel considers that the deposition pattern of zinc in chicken tibia and the nutritional equivalence of HMTBa from Mintrex® and from free HMTBa concerning the incorporation (as methionine) into body protein (Yi et al., 2007), are sufficient indications of an extended dissociation of the molecule. As zinc is the component of potential toxicological significance (see Section 6.1), its deposition in tissues and products represents the relevant issue for the appraisal of consumer safety.

The Scientific Committee on Food (SCF) established a tolerable upper intake level (UL) of zinc of 25 mg day⁻¹ (EC, 2003b). Mean intake values in Europe (excluding supplements) laid between 7.5 and 12 mg zinc day⁻¹. The 97.5 percentile in some countries (Austria, Ireland) was estimated to be higher than 20 mg and close to the UL, but this was not considered matter for concern by the SCF.

Currently, no comprehensive data are available to assess the contribution of foods from animal origin to total zinc intake by human population in the EU. In general, red meat is considered an important source of background zinc while milk is a food with low zinc content. In the Galicia region of Spain, the mean contribution of cattle meat products to the total daily zinc intake from meat products was reported to be 56 %; the contribution from foods of animal origin, mostly meat, to the total dietary zinc intake was indirectly estimated in the 50 % range (Alonso et al., 2002).

Zinc intake in vegetarians was found to be comparable to that in non-vegetarians, which suggests an equal contribution (i.e. 50 % each) from foods of animal and vegetable origin. Thus the background intake of zinc from foods of animal origin would be in the 4-6 mg day⁻¹ range, with the worst case being 11 mg day⁻¹.

In general, the chelate sources of trace elements are considered to have a higher bioavailability than inorganic sources –although this was not demonstrated in the studies provided by the applicant– and hence a potential to result in higher deposition in edible tissues. For a worst case calculation, a maximum 30 % higher bioavailability –in terms of tissue deposition– was assumed. A further refinement could be introduced because muscle tissue (meat), which represents around 50 % of total animal products, is unaffected by dietary zinc concentrations within the authorised limits. Therefore, based on the above assumptions, the additional contribution of zinc from the use of Mintrex®Zn in animal nutrition to consumer exposure is estimated not to exceed 1.7 mg day⁻¹.

---

6.3. Conclusions on safety for the consumers

The FEEDAP Panel retains zinc as the component of potential toxicological significance of Mintrex®Zn. According to a worst case estimation and with the support of intake data from the literature, the FEEDAP Panel concludes that the use of Mintrex®Zn in feed at the maximum authorised zinc levels is unlikely to cause a concern for the safety of consumers.

7. Safety for the user

No specific studies on the safety of Mintrex®Zn for the user were conducted. The product has a significant dusting potential: 10% of the particles are <105 µm, thus inhalation exposure may occur.

According to the assessment carried out by the Agency for Toxic Substances and Diseases Registry (ATSDR, 2005), inhaling large amounts of zinc (as dusts or fumes) can cause a specific short-term disease called metal fume fever. The long-term effects of high inhalation exposure are unknown. Low levels of zinc salts (acetate and chloride) cause skin irritation in rabbits, guinea pigs and mice. Although no clear human evidence exists, the ATSDR considers zinc acetate and zinc chloride as likely skin irritants in humans (ATSDR, 2005).

Therefore, the FEEDAP Panel supports the recommendations made by the applicant in the Register entry (under other provisions) and makes further recommendations (see Recommendations).

8. Safety for the environment

Zinc is a natural element that is essential for life and is present almost everywhere in the environment. However, at high concentrations zinc may be toxic and a maximum content of the element in feed has therefore been set. There is no reason to believe that chelates such as Mintrex®Zn would be more harmful to the environment than the inorganic element. The FEEDAP Panel considers that the use of Mintrex®Zn in feed does not represent additional risks to the environment, compared to other sources of zinc for which it will substitute, as long as the maximum authorised content in feedingstuffs is not exceeded.

9. Post-market monitoring

The FEEDAP Panel does not see a need for specific requirements of post-market monitoring other than the need for traceability and recall procedures established by Regulation (EC) No 183/2005.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Mintrex®Zn can be considered as a bioavailable zinc source, comparable to other authorised sources of zinc, for all animal species.

The FEEDAP Panel considers Mintrex®Zn as a new compound of trace elements which therefore requires demonstration of safety for the target species. The FEEDAP Panel considers one tolerance study necessary, performed on the most sensitive species. Since no specific studies on the tolerance of target animals to Mintrex®Zn were provided, the FEEDAP Panel cannot conclude on the safety of the compound for target animals.

---

20 OJ L 35, 8.2.2005, p.1
No data on edible tissues and products deposition were provided other than for liver from chickens and milk from dairy cows fed Mintrex\textsuperscript{®}Zn. Consequently, the FEEDAP Panel is unable to conclude on zinc deposition in other edible tissues and products. However, the FEEDAP Panel recognises that, from other published data, zinc in muscle tissue appears insensitive to zinc supplementation, within authorised levels, compared to liver and kidney. From the limited experimental data available, the FEEDAP Panel considers it unlikely that the use of Mintrex\textsuperscript{®}Zn would alter zinc deposition in edible tissues/products compared to other zinc sources.

Based on the data from acute toxicity and genotoxicity studies, the FEEDAP Panel considers that Mintrex\textsuperscript{®}Zn does not introduce any additional toxicity compared to other sources of dietary zinc.

With regards to consumer safety, the FEEDAP Panel retains zinc as the component of potential toxicological significance of Mintrex\textsuperscript{®}Zn. According to a worst case estimation and with the support of intake data from the literature, the FEEDAP Panel concludes that the use of Mintrex\textsuperscript{®}Zn in feed at the maximum authorised zinc levels is unlikely to cause a concern for the safety of consumers.

The FEEDAP Panel considers that Mintrex\textsuperscript{®}Zn is safe for the user provided that protective measures are taken.

The FEEDAP Panel considers that the use of Mintrex\textsuperscript{®}Zn in feed does not represent additional risks to the environment compared to other sources of zinc for which it will substitute.

**RECOMMENDATIONS**

The FEEDAP Panel recommends that the following modifications should be made to the Register entry as proposed by the applicant:

1. **Additive**
   In the view of the FEEDAP Panel the additive name is: Zinc chelate of hydroxy analogue of methionine.

2. **Description**
   Composition, description. Zinc chelate of hydroxy analogue of methionine containing a minimum of 16\% zinc and 80\% (2-hydroxy-4-methylthio)butanoic acid.
   Chemical formula. $\text{Zn(CH}_3\text{S(CH}_2\text{)}_2\text{-CH(OH)-COO)}_2$
   Purity Criteria. The FEEDAP Panel recommends the setting of maximum levels as follows: $\text{As} \leq 1$, $\text{Pb} \leq 5$, $\text{Hg} \leq 0.1$, $\text{Cd} \leq 1$ and $\text{F} \leq 0.2 \text{ mg kg}^{-1}$ Mintrex\textsuperscript{®}Zn. Dioxins (sum of PCDDs and PCDFs)$ \leq 0.2 \text{ ng WHO-PCDD/F-TEQ kg}^{-1}$ Mintrex\textsuperscript{®}Zn.

3. **Conditions of use**
   Feed formulations should be adjusted to account for the methionine efficacy of HMTBa in Mintrex\textsuperscript{®}Zn.
4. Other provisions and additional requirements for labelling

Specific conditions or restrictions for handling. In addition to the applicant’s proposal, the FEEDAP Panel recommends appropriate ventilation of working rooms and reduction of the inhalable fraction as far as technically feasible.

GENERAL REMARK

The FEEDAP Panel wishes to draw the attention to the following.

Considering:
- the increasing number of applications for organic forms of trace elements to be used in animal nutrition,
- the potential higher bioavailability of those compounds in feedingstuffs, and consequently (i) the potential reduction of maximum content for those trace elements in feed and (ii) the higher deposition of trace elements of organic origin in animal tissues, and
- the resulting need for maximum content of organic forms of trace elements, different from those already existing,

the FEEDAP Panel stresses the need for analytical methods to detect those organic compounds in the feed, independent from the trace element background. The availability of such methods would allow lower maximum contents in feed which would in turn reduce concerns relating to the assessment of consumer safety and reduce environmental load.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier Mintrex®Zn as nutritional additive for all species. March 2007. Submitted by Novus Europe SA.
5. Comments from the Member States received through the EFSAnet.

REFERENCES


APPENDIX

Executive Summary of the Evaluation Report of the Community Reference Laboratory Feed Additives on the Method(s) of Analysis of Mintrex® Zn for all species

Mintrex® Zn is a product for which authorisation is sought under the category "nutritional additives", functional group 3b "compounds of trace elements", according to the classification system of Annex I, of Regulation (EC) No 1831/2003. According to the applicant, Mintrex® Zn contains 16% of Zinc as chelate of hydroxyl analogue of methionine, 2-hydroxy-4-methylthiobutanoic acid (HMTBa) as active substance. Mintrex® Zn is also a source of methionine activity as HMTBa.

In the current application authorisation is sought for use of Mintrex® Zn for all animal species. Mintrex® Zn is intended to be added to complete feed to supplement Zn within legal limits for each species which are: pet animals 250 mg /kg, fish 200 mg/kg, milk replacers 200 mg/kg and other species 150 mg/kg.

For the determination of Zn in the feed additive, premixtures and feedingstuffs for official control the CEN standard method EN 15510:2007, as proposed by the applicant, is recommended by the CRL.

The proposed methods for the determination of HMTBa are considered suitable for the intended purpose.