

NANO PORT (USA) INC.

3380 Sheridan Drive Suite 262 Amherst, NY 14226 U.S.A
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February 9, 2004

Division of Standards and Labeling Regulations
Office of Nutritional Products, Labeling, and Dietary Supplements (HFS-820)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD, 20740-3835

Dear Sir,

FEB 23 2004
OB/FDA

New Dietary Ingredient Notification: Nano-Se

Nano Port (U.S.A.) Inc would like to introduce into the health food market of Nano Red Elemental Selenium (under the trade name of Nano-Se), following the 75 days waiting periods as provided by Law.

1. Distributor's name and address:

NANO PORT (USA) INC.
3380 Sheridan Drive Suite
262 Amherst
NY 14226, U.S.A.

2. Name of dietary ingredient:

Nano red elemental selenium under the trade name Nano-Se

3. Description of the dietary supplement that contains the dietary ingredient:

Each bottle contains 72 capsules

Each capsule contains 45 mcg selenium (Nano red elemental selenium)

Other ingredients: Starch and dextrin

Suggested usage: Take one to two capsules 1 to 2 times daily or as directed by a health professional.

4. Se and its Safety:

Selenium (Se) is an essential trace element, it is metabolised within the human body into an array of selenoproteins: classical glutathione peroxidase (GPx1), gastrointestinal glutathione peroxidase (GPx2), extracellular glutathione peroxidase (GPx3), phospholipid hydroperoxide glutathione peroxidase (GPx4), thioredoxin reductase (TR1 and TR2), iodothyronine deiodinase (IDI, IID1, and IID2), selenoprotein P, and selenoprotein W. It is well recognised that dietary selenium is important for a healthy immune response. There is also evidence that Se has a

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protective effect against some forms of cancer [1].

There are several selenocompounds in tissues of plants and animals. Selenate is the major inorganic selenocompound found in both animal and plant tissues. Selenocysteine is the predominant selenoamino acid in tissues when inorganic selenium is given to animals. Selenomethionine is the major selenocompound found initially in animal given this selenoamino acid, but is converted with time afterwards to selenocysteine. Selenomethionine is the major selenocompound in cereal grains, grassland legumes and soybeans. Se-methylselenocysteine is the major selenocompound in selenium enriched plants such as garlic, onions, broccoli florets and sprouts, and wild leeks. Sodium selenite is the major inorganic form for comparison of bioavailability and toxicity among different forms of Se [2, 3].

The typical American diet provides the average adult with about 80 to 150 mcg of Se per day, which is more than the newly revised RDA for selenium of 55 mcg, but less than one half of the amount considered optimal for utilization of the protective potential of Se, especially for cancer prevention. Accordingly, extra dietary selenium supplementation is increasingly recommended by health professionals. As to the safety of Se, a supplemental dose of 200 mcg per day would cause the total daily Se intake of an average adult to increase to 280 to 350 mcg. This is a safe amount since it is below or equal to the Reference Dose (RfD) for selenium, which, for an adult of 70 kg, was set by the EPA at 350 mcg [4,5]. The RfD is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. In line with this definition, studies have shown that prolonged daily selenium intakes of 750 to 850 mcg do not produce adverse effects [4, 5]. Sodium selenite, sodium selenate, selenomethionine, seleno-yeast and methylselenocysteine have been used for Se supplementation at the dose equal to or below 200 mcg Se daily. Long-term consumption of seleno-yeast (200 mcg Se daily, mostly in the form of selenomethionine) in 1312 persons for 4.5 years showed no toxicity and revealed a significant reduction in lung, prostate and colorectal cancer [6].

5. Nano-Se and its safety:

The efficacy of Se in inducing Se-containing enzymes and the pro-oxidative effect are determined by its chemical form. Normally, gray and black bulk particle of elemental Se (Se^0) has neither biological activity nor toxicity. It is known that particles of Se^0 formed from some bacterial strains and the redox system of glutathione or ascorbate and selenite has a very low bioavailability [7-9]. We observed that red elemental Se, formed in the redox system of selenite and GSH or other reducing agents, was unstable and could further aggregate into gray and black Se^0 if there were no controlling factors. We further found that protein existing in the redox system could

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affect the aggregation of red Se^0 . The resulting Se^0 was bright red, highly stable, soluble and of nano define size. Nano-Se was prepared by the reaction of bovine serum albumin (BSA), sodium selenite, and GSH under the Chinese Patent ZL97107038.5. The final solution containing Nano-Se and BSA. X-ray photoelectric energy spectra (XPS) showed the binding energy of Se 3d was 55.3 eV indicating Se^0 . Transmission Electron Microscopy (TEM) showed the size of red elemental Se was between 20~60nm [10, 11].

The Nano-Se shows totally different biological properties contrasting to the general concepts that elemental Se is inert. In HepG2 cells, Both Nano-Se and selenite have almost equal biological functions in increase of glutathione peroxidase (GPx), phospholipid hydroperoxide glutathione peroxidase (PHGPx) and thioredoxin reductase (TR), protection against free racial-mediated damage, and cell growth inhibition. Nano-Se has a 7-fold lower acute toxicity than sodium selenite in mice (LD_{50} 113 and 15 mg Se/kg body weight respectively). In Se deficient rat, both Nano-Se and selenite were efficient and generally equal in Se uptake and GPx biosynthesis [10].

Other toxicity investigations of Nano-Se are shown in two attachments, in general Nano-Se's subchronic toxicity is near to sodium selenite and Se-enriched soybean, however, sodium selenite and Se-enriched soybean at 6 ppm in diet caused more overt growth inhibition, haematology changes and transaminases release from liver compared with Nano-Se at the same Se dose in diet [attachment 1]. Although these observations could not lead to consider Nano-Se's subchronic toxicity is definitely lower than sodium selenite and Se-enriched soybean, however, it is safe to conclude that this novel form of Se is not more toxic compared with inorganic and natural occurring Se. An independent research using Nano-Se at 1, 3, and 6 ppm in diet for subchronic toxicity evaluation showed Nano-Se did not cause obvious growth inhibition, being consistent with the results in attachment1 [attachment2].

A new study definitely demonstrated that sub-chronic toxicity of Nano-Se was the lowest as compared with selenite and naturally occurring soy selenoprotein of Se-abudnant district [attachment3]. The major findings of this study were summarized here.

A) Nano-Se, sodium selenite and selenoprotein at high dose (5 ppm, 0.5 mg/kg bw) all decreased body weight gain in rats. The NOAEL of Nano-Se on body weight were 4 ppm (0.4 mg/kg bw), and the NOAEL of sodium selenite and selenoprotein on body weight were 3 ppm (0.3 mg/kg bw) for males and 2 ppm (0.2 mg/kg bw) for females, respectively, Therefore, the body weight gain decrease cause by Nano-Se was less strong than those caused by selenoprote in and sodium selenite.

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- B) Nano-Se, selenoprotein and sodium selenite at high dose all decreased hemoglobin, red cell count and platelet count in rats. The NOAEL of Nano-Se on hematology was 4 ppm (0.4 mg/kg bw) for males and 5 ppm (0.5 mg/kg bw) for females. The NOAELs of sodium selenite were 3 ppm (0.3 mg/kg bw) for males and females. The NOAELs of selenoprotein were 4 ppm (0.4 mg/kg bw) for males and 3 ppm (0.3 mg/kg bw) for females. Therefore, the decrease of hematology indicators caused by Nano-Se was less strong than those caused by selenoprotein and sodium selenite.
- C) Liver was the main target organ of the selenium compound toxicity which caused increased ALT and AST activities and decreased liver protein synthesis ability, thus decreasing TP and ALB level. The NOAEL of Nano-Se on clinical chemistry was 4 ppm (0.4 mg/kg bw) for both males and females. The NOAELs of sodium selenite were 3 ppm (0.3 mg/kg bw) for both males and females. The NOAELs of selenoprotein were 4 ppm (0.4 mg/kg bw) for males and 3 ppm (0.3 mg/kg bw) for females. Therefore, the toxicity of Nano-Se to liver was lower than selenoprotein and sodium selenite.
- D) Nano-Se, selenoprotein and sodium selenite at high doses increased the relative organ weight of rats. The NOAELs of Nano-Se on the relative organ weight were 4 ppm (0.4 mg/kg bw) for males and females. The NOAELs of sodium selenite were 3 ppm (0.3 mg/kg bw) for males and females. The NOAELs of selenoprotein were 3 ppm for males and 2 ppm (0.2 mg/kg bw) for females.
- E) Different degrees of liver lesions were observed in some rats of three selenium-treated groups, however, the degree of lesions induced by Nano-Se was significantly lower than sodium selenite and selenoprotein. The NOAEL of Nano-Se on liver histopathological changes was 3 ppm (0.3 mg/kg BW) and the NOAELs of sodium selenite and selenoprotein were 2 ppm (0.2 mg/kg BW). Kidney lesions were found in some rats of 5 ppm sodium selenite and selenoprotein-treated groups, while no kidney pathological changes were found in rats of Nano-Se-treated group.
- F) In conclusion, if the effect of selenium on the growth, hematology and clinical chemistry was assessed comprehensively, the NOAEL of Nano-Se was 4 ppm (0.4 mg/kg bw) for both male and female rats, and the NOAELs of sodium selenite and selenoprotein were 3 ppm (0.3 mg/kg bw) for males and 2 ppm (0.2 mg/kg bw) for females. If the effect of selenium on the histopathological changes of liver was used as the end point, the NOAEL of Nano-Se was 3 ppm (0.3 mg/kg bw) for both male and female rats, and the NOAELs of sodium selenite and selenoprotein were 2 ppm (0.2 mg/kg bw) for males and females. All in all, the toxicity of selenium from Nano-Se was lower than sodium selenite and selenoprotein.

Nano-Se, taken at the dose of 180 mcg Se daily, was granted as health care food by Ministry of Hygiene P. R. China in 1998.

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In summary, Nano-Se has comparable bioavailability of selenite, sharply lower acute toxicity, lower sub-chronic toxicity. Supplementation at 180 mcg per day for adults is within the scope of RfD. Nano-Se is the safest Se form for supplementation based on the present available research results.

Dr. JS Zhang is our Research Scientist and would appreciate any comment you may have on the Nano-Se product prior to its introduce into the health food market.

If more information is needed, please let us know.

6. Signature of the distributor of this dietary supplement.

NANO PORT (USA) INC.

By:

A handwritten signature in black ink, appearing to read 'Yu Har Fei', is written over a horizontal line. The signature is stylized and cursive.

Yu Har Fei
President

Enclosures

Reference

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10. JS Zhang, XY Gao, LD Zhang, and YP Bao. Biological effects of a nano red elemental selenium. *BioFactors.* **15**:27-38, 2001.
11. XY Gao, JS Zhang, and LD Zhang. Hollow sphere selenium nanoparticles: their in vitro anti hydroxyl radical effect. *Adv. Mater.* **14**:290-293, 2002.

Attachments

1. Report on toxicity test of Xiwang capsule
2. REPORT ON QUALITY TEST by Analytic and Testing Centre of Nanjing Railway Medical College
3. Report on a 90-day subchronic toxicity test on Xiwang capsule (Nano-Se) in rats