



American Institute for Biosocial and Medical Research, Inc.
Natural and Medicinal Products Research

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N-Acetyl-L-Hydroxyproline

New Dietary Ingredient Notification



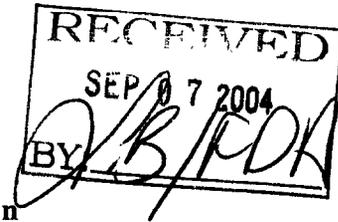
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September 2, 2004

Dr. Susan Walker,
Director, Division of Dietary Supplements,
Office of Nutritional Products, Labeling and Dietary Supplements, HFS 810,
Center for Food Safety and Applied Nutrition,
Food and Drug Administration
5100 Paint Branch Pkwy
College Park, MD 20740-3835



**New Dietary Ingredient Notification
for N-Acetyl-L-Hydroxyproline**

Dear Dr. Walker,

We have been authorized to submit a premarket New Dietary Ingredient Notification on behalf of Kyowa Hakko Kogyo Co., Ltd., as required under 21CFR190.6.

Our street mailing address for communications is:

Attention: Richard Conant
V.P. Technical and Regulatory Affairs
Life Sciences Division,
AIBMR Life Sciences, Inc.,
4117 S. Meridian,
Puyallup, WA 98373
Phone: 253-286-2888
FAX: 253-286-2886

As specified in the Code of Federal Regulations, Chapter 21 CFR, Part 190-Dietary Supplements; Subpart B, Paragraph 190.6 (revised as of April 1, 2002) for Requirement for Premarket Notification we are submitting information about Kyowa Hakko Kogyo Co., Ltd.'s ingredient product **N-Acetyl-L-Hydroxyproline**.

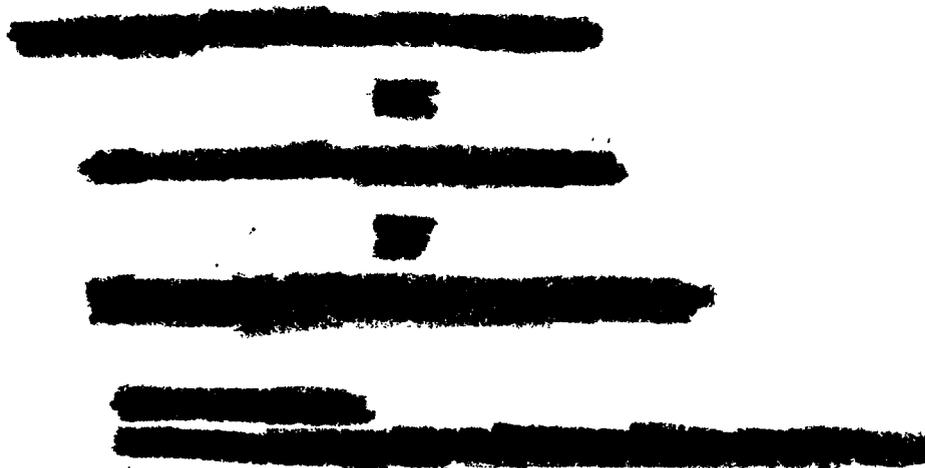
This notification will present evidence that **N-Acetyl-L-Hydroxyproline** (hereinafter AHYP), proposed herein as a new dietary ingredient, when used under the suggested intake recommendations and labeling directions is reasonably expected to be safe. Following the format of the CFR notification requirements for paragraph (b) this notification will supply answers for the requested details as follows.

(1) **Name and address of manufacturer:** Kyowa Hakko Kogyo Co., Ltd.
Bio-Chemicals Company
1-6-1 Ohtemachi
Chiyoda-ku, Tokyo 100-8185
Japan

Manufacturing site: Kyowa Hakko Kogyo Co., Ltd.
1-1 Kyowa-machi
Hofu-shi, Yamaguchi 747-8522
Japan

(2) **The name of the new dietary ingredient: N-Acetyl-L-Hydroxyproline.**
N-Acetyl-L-Hydroxyproline is a derivative of a natural amino acid, L-Hydroxyproline, which is found in collagen, a most abundant protein in the human body. Kyowa Hakko's L-Hydroxyproline is produced by fermentation and free from potential risk of BSE.

Flow Chart of the production process:



(3) **A description of the dietary ingredient:**

- (i) The level of the new dietary ingredient in a dietary supplement will be in the range of 50 mg to 100 mg per tablet or capsule.
- (ii) The conditions of use are daily intakes of not more than 300 mg with single 50 mg to 100 mg servings taken three times per day.

As the agent for the manufacturer of the ingredient, Kyowa Hakko U.S.A., Inc. will responsibly submit the following outline of label directions to dietary supplement manufacturing and marketing customers.

Statement of identity:

e.g. "N-Acetyl-L-Hydroxyproline"

Net quantity of contents:

e.g. "60 capsules"

Structure-function claim and label wording:

A dietary supplement for healthy joints*

*This statement has not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease.

"This product is not intended for use by pregnant women and children. This product should be taken for a maximum period of 8 months. If you take any medication, consult with a physician before using this product."

Directions for use:

e.g. "Take three capsules daily."

(Daily intake not to exceed 300 mg of N-Acetyl-L-Hydroxyproline)

(4) The history of use and the evidence of safety establish that AHYP, when used under the aforementioned conditions, is reasonably expected to be safe.

Immediately following herein we summarize information about the safety data on AHYP reported in nonclinical animal studies, *in vitro* tests, and from human clinical studies. The previous LD₅₀ and the most recent GLP limit test data suggest AHYP has no acute toxicity even at a possible erroneous or accidental intake of a high amount of AHYP in a short term period. AHYP's non-mutagenicity and non-chromosome aberration activity shown by the *in vitro* study reflect the fact that there has been no report or association of carcinogenicity reported from clinical reports that span a period over twenty years of use.

The mechanism of action of AHYP is to help promote proteoglycan synthesis in normal, healthy cartilage.

Safety Data (Nonclinical (animal) and *in vitro*)

There are three LD_{50} studies by oral administration. These studies reported the following outcomes:

- 7,451 mg/kg of body weight (rats)¹
- 5,688 mg/kg of body weight (mice)¹
- >2,500 mg/kg of body weight (rats)¹²

A recent GLP compliant acute oral toxicity study (limit test) with 14-day post-treatment observation period and gross pathology reported the following outcomes²:

A single oral dose of 2,000 mg/kg body weight of AHYP (Kyowa Hakko; lot number: 0145003) was administered to rats orally by gavage. Animals were observed for lethality and toxic symptoms for 14 days. Gross pathological examination was carried out on the 15th day. The outcomes of this study were reported as follows:

- 1) No deaths occurred after oral administration of N-acetyl-L-hydroxyproline at the 2,000 mg dose.
- 2) No toxic clinical symptoms were observed.
- 3) Scheduled autopsy carried out on day 15 revealed no gross pathological changes.
- 4) No adverse effects were noted at a single oral dose of 2,000 mg/kg N-acetyl-L-hydroxyproline in male and female rats.

Another recent GLP compliant subchronic (90-day) oral toxicity study with histopathological examination of AHYP in rats (Kyowa Hakko; lot number: 0145004) (four groups of 20 male and 20 female Sprague-Dawley rats received daily oral doses of 0 (control), 40, 200 or 1000 mg/kg by gavage for 3 months, 7 days per week) reported the following outcomes.³

- 1) No mortality occurred.
- 2) No treatment-related clinical symptoms were observed.
- 3) No difference was observed in body weights compared to the control throughout the study period. Food and water consumption was similar to the control.
- 4) No pathological changes were observed in the eyes of the 1000 mg/kg treated male and female animals.
- 5) No treatment related changes were observed in sensory reactivity, grip strength and motor activity.
- 6) No changes were observed in hematological parameters or prothrombin time. A dose-related decrease in SGOT/AST, SGPT/ALT and AP was observed and assessed as having no direct toxicological meaning. No changes in other serum chemistry parameters or urinalysis were observed.
- 7) No treatment-related gross pathological lesions occurred in organs of animals receiving N-acetyl-L-hydroxyproline at doses up to 1000 mg/kg for three

months. The absolute thymus weight decreased slightly in males only; all other organ weights remained unchanged.

- 8) No treatment-related gross or histopathological lesions occurred in the organs of rats administered the 1000 mg/kg dose of N-acetyl-L-hydroxyproline for three months.
- 9) N-acetyl-L-hydroxyproline was well tolerated in daily oral doses up to 1000 mg/kg.
- 10) The no observed adverse effect level in the rat is lower than 1000 mg/kg.

In a previous 29-day oral administration study of AHYP (4.5; 36; 288 mg/kg of body weight) in rats, no unwanted effects occurred.¹

In another previous 28-day study via oral administration of AHYP (4.5; 36; 288 mg/kg of body weight) in dogs, no unwanted effects occurred other than slight changes on the cornea and renal tubules of some animals.¹

Mutagenicity

No indications of any mutagenic potential were found on the Ames Test: negative, up to 5000 µg/plate (maximum dosage examined).¹³ For the Chromosome Aberration Test the results were negative up to 1730 µg/ml (maximum dosage examined).¹⁴

Carcinogenicity

From bioassays and reports from clinical studies, no indications of a tumorigenic potential have been reported. No reproducible teratogenic effects were observed in rabbits with the dosage of 288 mg/kg of body weight.¹

Metabolism/Excretion

Ingested AHYP is shown to be completely excreted from the body within a couple of days without being metabolized in dogs.¹⁰

As discussed above, an important aspect of short-term and long-term safety of AHYP is that AHYP has been demonstrated to be unmetabolized. Once AHYP is ingested, it is absorbed and moves to the blood stream. The half-life of AHYP in the blood stream is only several hours and without being metabolized, 100 % of AHYP is excreted from the body. Therefore, AHYP does not accumulate in any part of the body, nor does it produce any deleterious metabolites that should remain in the body for a long term.¹⁰

Safety Data (Humans)

600 mg/day

48 subjects (n=48), 8 weeks.
No incidence of non-tolerance reported.⁴

32 subjects (n=32), 6 weeks.
No side effects were observed.⁵

50 subjects (n=50), 8 months.
No side effects were observed.⁶

100 subjects (n=100), 15 days to 6 months.
No incident on any of the cases regarding digestive, hepatic, renal tolerance.⁹
No influence of the cardio-vascular and central or autonomous nervous system.⁹

1200 mg/day (14 days) followed by 600 mg/day for 6 weeks

367 subjects (n=367)
No serious adverse reactions were noticed.
Occasional transient gastrointestinal complaints (3.52%)⁷.

509 subjects (n=509)
Low degree of side effects were noted (4.7%) mainly on the gastrointestinal tract of a transient nature.⁸

1200 mg/day (21 days)

132 subjects (n=132)
No serious adverse events were reported. (18.9% of total had non-serious transient side-effects; 11.4% patients had side-effects considered related to treatment or non-classified as: 3 cases of gastrointestinal discomfort, 3 cases of nausea, 2 cases of skin rash and 2 cases of constipation.)¹¹

Open Multicenter Study (600 mg/day)¹⁵

5,523 subjects (n=5523), 4 and 8 weeks.
2.79% (2.3% gastrointestinal complaints, 0.27% allergic reactions, 0.22% other).
98.8%, reported "very good" or "good" tolerance.

In the clinical study, AHYP is regarded as a well-tolerated and safe substance. However, as is reported in the reference #11, the percentage of side effect (SE) occurrence at the dosage of 1200 mg/day is not negligible. The SE incidence can be judged as dose dependent

in the range of between 600 mg/day and 1200 mg/day, as reported in the literature. At the generally prescribed dosage of 600 mg/day, the incidence of SE ranged between zero^{4,5,6,9} to 4.7%⁸, while at 1200 mg/day the figure increased to 18.9%¹¹, the vast majority of which included transient gastrointestinal complaints.

The incidence of side effects in these trials requiring discontinuation of the product is less than 1%, ranging between 0.05% to 0.97%. Drop-outs occurred only at the 1200 mg daily dose. In one trial at that dose (Ref. #11), no drop-outs were reported. These findings, together with the complete absence of hispathologies observed in the subacute oral toxicity study of N-acetyl-L-hydroxyproline in rats (Ref. #3), at three different sub-acute doses for a subchronic period of 90 days, allometrically equivalent to one and one-half years intake in humans, support the position that AHYP, at the 300 mg daily dose specified in the conditions of use for the product, for a maximum of 8 months, can reasonably be expected to be safe.

Although reference #15 showed 2.79% SE in 8 week study, references #6 and #9 reported no side effects in 8 months and 6 months studies, respectively, suggesting that the incidence of SE does not increase for over 8 weeks of AHYP intake. In other words, if the SE frequency were dependent on intake duration for over 8 weeks, the studies in references #6 and #9 should have resulted in significantly higher SE frequency than 2.79% (reference #15), but that was not the case. This led to the conclusion that, in addition to limiting the daily intake of AHYP to 300 mg/day, fixing the recommended duration of AHYP intake to 8 months will mitigate the risk of SEs with the use of AHYP for a period longer than the duration of the clinical studies cited.

From the comparison of the SE frequencies between the two intake levels (600 mg/day and 1200 mg/day), setting the recommended intake of AHYP at 300 mg/day assures a significant reduction of SE frequency at the lowest order of toxicity based on the data from the nonclinical and clinical studies reported above.

Based on these views, we determined AHYP's use as an ingredient for dietary supplements as follows. These instructions will provide significant guidelines to reinforce the safe use of AHYP as a dietary supplement ingredient.

- 1) AHYP is intended to be used as an ingredient in dietary supplements that help maintain healthy joints.
- 2) The recommended intake of AHYP is up to 300 mg/day. Each label of a dietary supplement containing AHYP will show directions for use based on the 300 mg maximum daily intake.
- 3) Labels of dietary supplements containing AHYP will display the following warnings:
 1. "This product is not intended for use by pregnant women and children."

14. Wright NP, Nolan S: N-Acetyl-L-Hydroxyproline: Chromosome Aberration Test in CHL Cells *in Vitro*. SPL Project Number: 732/092 (2001).
15. Franz M: Die Behandlung degenerativer Gelenkerkrankungen mit Oxaceprol. *Rheuma*, 12, 139-143 (1992).

Enclosures (in 3-ring binder)

2. "This product should be taken for a maximum period of 8 months."
3. "If you take any medication, consult with a physician before using this product."

Sincerely,



Richard Conant
Vice President
Technical and Regulatory Affairs

REFERENCES

1. Product description of AHYP, issued by Chephasaar Chem.-Pharm. Fabrik GmbH., St. Ingbert, Austria.
2. Acute oral toxicity study of N-acetyl-L-hydroxyproline with 14-day post-treatment observation period in the rat (limit test). Final Report, #PDCL 0402 (2004).
3. Subchronic (90 day) oral toxicity study of N-acetyl-L-hydroxyproline in rats. Final Report, #PDCL 0403. (2004).
4. Schubotz R et al. Treatment of osteoarthritis with N-acetyl-hydroxyproline. *Therapiewoche* 27, 4248 (1977).
5. Finkbeiner G et al. Conservative gonarthrosis therapy with Oxaceprol. *Therapiewoche*, 36(2), 116 (1986).
6. Michael J et al. Therapy of coxarthrosis and gonarthrosis with Oxaceprol. *Therapiewoche*, 36(29), 3076 (1986).
7. Diehl K et al. Therapy of chondropathia patellae with Oxaceprol. *Therapiewoche*, 35(28), 3388 (1985).
8. Diehl K et al. Therapy of gonarthrosis with Oxaceprol. *Therapiewoche*, 35(1), 51 (1985).
9. Laborie G et al. The acetyl-hydroxyproline, metabolic harmonization of connective tissue (100 cases in rheumatologic practice). *Bordeaux Medical*. 3, 3059 (1970).
10. Lachmann G et al. Pharmacokinetics and metabolism of ¹⁴C-Oxaceprol in beagle dogs after intramuscular and oral administration. *Arzneimittel-Forschung*, 40, 200-6 (1990).
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12. Dreher DM. N-Acetyl-L-Hydroxyproline: Acute Oral Toxicity In the Rat-Acute Toxic Class Method. SPL Project Number: 732/093 (2001).
13. Bowles AJ: N-Acetyl-L-Hydroxyproline: Reverse Mutation Assay "Ames Test" Using *Salmonella Typhimurium* and *Escherichia Coli*. SPL Project Number: 732/091 (2001).