

November 25, 2009

Dr. Barbara Shane  
Executive Secretary for the Board of Scientific Counselors  
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National Institute of Environmental Health Sciences  
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Dear Dr. Shane,

The following comments are submitted on behalf of the more than two million members and supporters of People for the Ethical Treatment of Animals (PETA) and the Physicians Committee for Responsible Medicine (PCRM) in response to the nominations of substances to NTP for study in 2009 (October 23, 2009; Federal Register 74(204):54821). Our organizations are committed to using the best available science to protect animals from suffering and to promote the acceptance of human-relevant methods for risk assessment.

Specific comments are submitted for butterbur (*Petasites hybridus*) extract, evening primrose oil (*Oenothera biennis* L.) extract and valerian (*Valeriana officinalis* L.) root extract. NTP has recommended additional animal tests for these substances that would result in the poisoning and death of thousands of animals if carried out. In each case, we urge NTP to thoroughly consider human experience, existing toxicity data and the application of non-animal test methods in order to avoid unnecessary and duplicative animal tests.

We are especially concerned that these substances are all herbal dietary supplements with long histories of safe use and with little reason for concern regarding potential toxicity. While we understand FDA's constraints in regulating dietary supplements, the potential toxicities of these particular substances is clearly suited to more relevant investigation by clinical trials and post-marketing surveillance studies. The unnecessary and irrelevant animal testing programs proposed waste time, resources and animals' lives.

Thank you for your attention to these comments. I can be reached at (757) 622-7382, ext. 8001, or by e-mail at [josephm@peta.org](mailto:josephm@peta.org).

Sincerely,

[ Redacted ]<sup>n</sup>

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## **Butterbur (*Petasites hybridus*) extract**

Butterbur was nominated by NIEHS for comprehensive toxicological characterization based on its widespread use as a dietary supplement, suspicion of contamination with toxic pyrrolizidine alkaloids, and lack of toxicity data on other constituents. In addition, NTP states in its research concept that there is insufficient data to determine if there is additional risk of adverse effects in pregnant or nursing mothers, especially with evidence from one clinical case report of fetal death as the result of maternal consumption of butterbur.

As with the other herbal dietary supplements nominated, evening primrose oil and valerian, human experience and existing toxicological data do not support a high level of concern for butterbur's potential toxicity. As noted in the review document, butterbur has been used since the 17<sup>th</sup> century to treat a variety of medical conditions. In a postmarketing surveillance study of 580 patients treated for allergic rhinitis with a commercially available butterbur extract in Switzerland, Käufeler et al. (2006) found the overall incidence of adverse events to be very low and similar to the occurrence of adverse events in the placebo group (primarily gastrointestinal disorders). The authors concluded that butterbur extract was confirmed by three Good Clinical Practice trials and two post-marketing surveillance trials to be safe and efficacious. Similarly, Guo et al. (2007) conducted a systematic review of five databases to evaluate the quality of the available studies on butterbur and other herbal medicines for the treatment of allergic rhinitis and concluded that most trials found good tolerability and similar adverse events compared with the control interventions.

Regarding the clinical case report of a fetal death as the result of maternal consumption of butterbur, it is important to note that the mother consumed large amounts of herbal tea, which contained PAs, during pregnancy. As stated in the review document, PA metabolites are known to be hepatotoxic. Current processing methods using CO<sub>2</sub> or propane yield butterbur extracts that contain PAs below the detection limit and most commercial products state that the preparations are PA-free or contain no detectable PAs. This known source of potential toxicity can therefore be effectively controlled by analytical methods and appropriate labeling of available butterbur products.

It is also noteworthy that the review document references an unpublished 26-week toxicity study in rats from 2001 by Weber & Weber GmbH & Co. KG, Inning, Germany, manufacturers of the commercial butterbur preparation Petadolex®. This study is described by Danesch and Rittinghausen (2003), employees of Weber & Weber, as a chronic toxicity study performed in 200 Wistar rats for a period of 26 weeks that was conducted in accordance with ruling EEC Directives, with the ICH Guideline "Duration of Chronic Toxicity Testing in Animals" and with the OECD Guideline for Testing of Chemicals. These authors state further that Principles of Good Laboratory Practice (GLP) as specified by national and international legislation were strictly followed, and that a no adverse effect level (NOAEL) could be established for the lower dose range tested well above and at an adequate safety distance of the recommended dose in humans. Every

effort must be made to obtain and thoroughly review this apparently highly relevant study rather than initiating potentially repetitive animal tests (Anonymous, 2001).

In summary, butterbur's long history of safe use in humans and the lack of serious adverse effects reported in safety and efficacy trials indicate a low level of concern for the potential toxicity of butterbur products prepared by current extraction methods. The potential toxicity resulting from PA contaminants should be addressed by analytical methods and appropriate labeling of butterbur products rather than by unnecessary and repetitive animal testing. As with the other herbal dietary supplements nominated, butterbur's effects would be investigated more relevantly in human studies.

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### **Evening primrose oil (*Oenothera biennis* L.) extract**

Evening primrose oil (EPO) was nominated for toxicological characterization by NIEHS because of its widespread use in dietary supplements, lack of adequate toxicological data, and concern regarding potential adverse effects among populations that use EPO regularly. In particular, NTP states in its research concept that the pattern of use and reported effects on reproductive endpoints suggest that additional studies in this area would be warranted. NTP also proposes an immunotoxicity screening study to “clarify” the immune functions and cell populations modulated by EPO.

As noted for butterbur, NTP’s concern for the potential toxicity of EPO appears to be greater than the evidence warrants. The entire evening primrose plant is edible. Native American tribes consumed it as a staple food and used it to treat a variety of ailments (Artz, M.B., 2007). EPO consists mainly of essential fatty acids including linoleic acid,  $\gamma$ -linolenic acid, oleic acid and palmitic acid. These are normal intermediates in human metabolism and are therefore very unlikely to be inherently toxic. In a 1992 review, Horrobin reported that, of 4000 patients involved in clinical trials of EPO for three months or more at doses of 3-6 g/day, not a single adverse event occurred significantly more frequently in patients receiving EPO than in those receiving placebo. The studies reviewed included one in which 14 children with cystic fibrosis received doses of up to 20 g/day for one year. Horrobin also noted that about half a million prescriptions for EPO had been dispensed by the UK National Health Service for the treatment of atopic eczema or breast pain and that the incidence of reported adverse events had been far lower than with most drugs with no pattern suggesting that any adverse events were related to its use. Long term studies in four species of animals in which reproductive toxicity, teratogenicity and carcinogenicity were evaluated likewise found no toxic effects attributable to EPO.

With regard to effects on reproductive endpoints, NTP claims that labor lasted longer in women taking EPO than in those who did not, citing a retrospective study by Dove and Johnson (1999). However, the study’s authors state that they could not conduct a valid analysis of variance to determine whether differences in length of labor existed because they observed a wider variation in length of labor in the EPO group. In any case, it is unclear how this particular endpoint could be evaluated in the proposed reproductive toxicology study in rats. NTP also claims that the use of EPO was associated with increases in active phase labor abnormalities including incidence of cesarean delivery and vacuum extraction. However, the study’s authors noted no effect on cesarean delivery in their discussion and expressed uncertainty over the significance of a marginal increase in vacuum extractor use. They also note that their study was limited by its retrospective design which used client records to assess outcome variables. There was no strict procedure for defining onset of labor or latent phase and no attempt was made to control for other variables, including the use of homeopathic remedies or castor oil, which might independently influence cervical ripening or labor onset. They identify a need for replication of their study with a larger, more controlled sample.

NTP proposes to conduct a reproductive toxicology based on what it describes as “the demonstrated evidence in the literature of effects on reproductive endpoints in humans and experimental animals.” However, NTP itself cites Leaver et al., (1986) who show that Wistar rats fed a diet supplemented with a commercial EPO preparation from three weeks of age until mating showed no differences in parturition, birth weight, postnatal growth rate, maternal weight during pregnancy, and fetal or placenta prostaglandin E2 levels as compared to control animals. Effects on male reproductive function in ICR mice orally administered EPO, including increases in testis weight and the number of complete penile insertions during a three-hour period, reported in a CAB abstract, were apparently not regarded as adverse effects by the study’s authors who titled their work “Improving effect of evening primrose oil on the sexual functions of male mice” (Shin, S.J., and Lee, J.H. 2006)

NTP proposes an immunotoxicity screening study to “clarify” the immune functions and cell populations modulated by EPO and inform selection of additional models that may be of value in evaluating susceptibility. As noted above, EPO consists of polyunsaturated fatty acids some of which have been shown to have immunomodulatory effects. Existing and ongoing investigations of the effects of these individual constituents are applicable to determining the potential immunotoxicity of EPO as a whole. In any case, since its constituents are normal metabolic intermediates and dietary components, more relevant data would be obtained from studying the effects of EPO with human volunteers than in animals.

In summary, EPO’s long history of safe use in humans as food and medicine, its chemical composition of essential fatty acids normally occurring as metabolic intermediates and the lack of serious adverse effects reported in clinical trials or as a result of its prescribed uses indicate a low level of concern for its potential toxicity. As with butterbur, any further investigation should be assigned a low priority and preference should be given to studies of effects in humans.

## References

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### **Valerian (*Valeriana officinalis* L.) root extract**

Valerian was nominated by NIEHS for comprehensive toxicological characterization based on its widespread use in dietary supplements, the lack of adequate toxicological data, and concerns regarding potential adverse developmental and reproductive effects.

As noted for the other herbal dietary supplements nominated, NTP's concern for the potential toxicity of valerian does not appear to be supported by human experience and existing data. Valerian has been used to treat digestive and urinary tract problems for over 1000 years and is also widely used as a mild sedative and sleep aid. German Commission E recommends valerian for use in the management of restlessness and nervous disturbances of sleep (Yao, M., et al. 2007). In addition, no serious adverse effects were reported in a recent review of 37 clinical trials by Taibi et al. (2007). The most common side effects were mild dizziness, headache, drowsiness or gastrointestinal discomfort. The authors concluded that the studies reviewed support the safety of valerian.

Regarding potential adverse developmental and reproductive effects, NTP states in its research concept that there are two studies suggesting that valerian extracts retard ossification in rats and mice following developmental exposures. However, it appears that only Tufik et al. (1994), using a mixture of valepotriates, reported statistically significant increases in the number of retarded ossifications – at doses ten to twenty times greater than those prescribed for humans. No additional details of these observations were presented. In a more recent study using a valerian extract in ethanol, Yao et al. (2007) reported no significant difference in the number of ossified sternebrae in fetuses from any of the treatment groups at doses up to 65 times greater than the normal human dose. While the authors observed differences in the number of ossified metacarpals, similar differences were also found in the ethanol control group compared to the water control group. In addition, no significant differences were found in the mean number of implantations per dam, corpora lutea per dam, live fetuses per litter, total number of resorptions or percentage pre-implantation loss compared to ethanol or water control groups and no external malformations were observed. The authors concluded that valerian extract did not have any significant adverse effects on fertility or embryo development.

NTP also states that increases in the frequency of micronuclei in polychromatic erythrocytes in the femur observed in mice receiving a valerian extract at 500 – 2000 mg/kg/d suggest that some of the constituents of valerian may be genotoxic. In fact, these increases were only observed by Al-Majed et al. (2006) at the highest dose – 39 times greater than the recommended human dose. In addition, the authors acknowledge that the depletion of testicular nucleic acids observed at higher doses was not dose-dependent and that their data contradict a substantial body of literature showing that many of valerian's constituents are found to be antioxidants known to protect against genotoxicity and/or carcinogenicity.

NTP states that there are six case reports of hepatotoxicity but that since these patients were taking mixtures of herbal supplements containing valerian and other herbs, the contribution of valerian to the hepatotoxicity was uncertain. In its review document, NIEHS notes that researchers now believe that germander, a plant from the mint family and also believed to be present in the herbal medicines, was the cause of the liver damage and that in several recent reviews and meta-analyses of the efficacy of valerian involving more than 1000 patients, no occurrence of hepatotoxicity was reported. Also, in a review of 23 cases of self-poisoning with a valerian-containing insomnia treatment commercially available in Hong Kong, Chan et al., (1995) found no clinical evidence of acute hepatitis and no evidence of subclinical liver damage in twelve patients on whom routine liver function tests were performed.

Regarding the proposed approach, we are concerned that uterotrophic assays could be employed in choosing the test material. The only evidence for valerian's estrogenic activity appears to be from a survey of seventeen different plant extracts which produced conflicting results for transcriptional activity and receptor binding *in vitro* (Overck, C.R., et al. 2008). The proposed *in vitro* screens to evaluate estrogenic activity should be regarded as definitive and negative results should not be taken as a compelling reason to try uterotrophic assays. In addition, an *in vivo* micronucleus assay is proposed in the first tier of toxicity testing. Since conflicting *in vitro* mutagenicity results have been reported, these *in vitro* tests should be repeated and *in vitro* tests should also be employed to investigate valerian's potential to induce chromosomal aberrations in the first tier of testing.

In summary, valerian's long history of safe use in humans, the lack of serious adverse effects reported in numerous safety and efficacy trials, and the lack of reliable and reproducible effects reported in existing toxicology studies indicate a low level of concern for its potential toxicity. As with the other herbal dietary supplements nominated, any further investigation should be assigned a low priority and preference should be given to studies of effects in humans.

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