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Safety of docosahexaenoic acid (DHA) administered as DHA ethyl ester in a 9-month toxicity study in dogs

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ABSTRACT

DHA Ethyl Ester (DHA-EE) is a 90% concentrated ethyl ester of docosahexaenoic acid manufactured from the microalgal oil. The objective of the 9-month study was to evaluate safety of DHA-EE administered to beagle dogs at dose levels 150, 1000 and 2000 mg/kg bw/day by oral gavage and to determine reversibility of any findings after a 2-month recovery period. DHA-EE was well tolerated at all doses. There were observations of dry flaky skin with occasional reddened areas at doses ≥1000 mg/kg bw/day. These findings lacked any microscopic correlate and were no longer present after the recovery period. There were no toxicologically relevant findings in body weights, body weight gains, food consumption, ophthalmological examinations, and ECG measurements. Test article-related changes in hematology parameters were limited to decreases in reticulocyte count in the high-dose males and considered non-adverse. In clinical chemistry parameters, dose-related decreases in cholesterol and triglycerides levels were observed at all doses in males and females and attributed to the known lipid-lowering effects of DHA. There were no effects on other clinical chemistry, urinalysis or coagulation parameters. There were no abnormal histopathology findings attributed to test article. The No-Observable-Adverse-Effect Level of DHA-EE was established at 2000 mg/kg bw/day for both genders.

1. Introduction

Docosahexaenoic acid (DHA, C22:6n-3) is a long-chain omega-3 polyunsaturated fatty acid. DHA-EE is a highly concentrated ethyl ester of DHA (DHA-EE) containing 90% DHA by weight (900 mg/g). DHA is derived from the heterotrophic microalga *Crypthecodinium cohnii* in a controlled fermentation process, and the resulting triglyceride oil (DHA single-cell oil [DHASCO®]) is extracted and refined according to current good manufacturing practices. DHASCO® oil, which naturally contains 480–600 mg/g DHA, then undergoes transesterification and purification to 90%.

The described herein 9-month toxicity study in male and female beagle dogs was a part of an extensive toxicological evaluation of DHA-EE including subchronic and chronic toxicity studies in rodent and non-rodent species. In the previously published 90-day rat study, the no-observable-adverse-effect level (NOAEL) for DHA-EE was determined at 2500 mg/kg bw/day (Hadley et al., 2010). In the 26-week rat study, administration of DHA-EE was well tolerated at levels up to 2000 mg/kg bw/day, the highest dose tested, as there were no notable clinical observations, no changes in body weight or in clinical chemistry parameters, and no histopathological findings other than an increased number of sinus histiocytes in the mesenteric lymph nodes of males at ≥1000 mg/kg bw/day and females at ≥500 mg/kg bw/day. This finding was considered minimal and non-adverse. Therefore, based on these results, the NOAEL for the 26-week study was considered to be 2000 mg/kg bw/day in both male and female rats (Bonnette, 2012).

DHA-EE was tested in a battery of genetic toxicity assays including a bacterial reverse mutation test (Ames test), a chromosome aberration test in human lymphocytes and an *in vivo* micronucleus test in a rat bone marrow. All tests were negative, indicating lack of DHA-EE genotoxic activity in tests for induction of mutations and/or chromosome damage when tested in accordance with regulatory guidelines (unpublished reports, on-file with DSM).

Further, potential effects of DHA-EE on hemodynamic and electrocardiographic parameters via telemetry was evaluated in beagle dogs following an oral administration of DHA-EE at doses up to 2000 mg/kg bw. No test article-related effects were noted on the

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hemodynamic (systemic blood pressures and heart rate) and electrocardiographic parameters (PR, QRS, QT and QTc intervals) following the administration of DHA-EE (unpublished reports, on-file with DSM).

In addition, the impact of DHA-EE on the rat respiratory system following an oral administration at doses up to 2000 mg/kg bw was investigated. No effects were noted on the respiratory rate, minute volume and tidal volume of rats demonstrating DHA-EE safety for the respiratory system (unpublished reports, on-file with DSM).

The 9-month dog study was preceded by a dose-range finding 8-week study in beagle dogs. The 8-week study investigated tolerability and toxicity of DHA-EE when administered via oral gavage at doses of 0, 100, 500, 1000, and 2000 mg/kg bw/day. DHA-EE was well tolerated at all doses and resulted in increases in DHA levels in plasma and the brain at the study termination. DHA-EE administration at doses ≥500 mg/kg bw/day was associated with dry flaky skin; doses of 1000 and 2000 mg/kg bw/day were also associated with yellow discoloration of the fur. These findings were not considered to be adverse and the NOAEL was considered to be 2000 mg/kg bw/day for both male and female dogs (Raineri, 2011).

The objective of the herein described chronic study (Bonnette, 2011) was to evaluate the potential toxicity and toxicokinetics of DHA-EE when administered orally for 9 months (273 days) at doses of 0, 150, 1000, and 2000 mg/kg bw/day and to determine the persistence, reversibility, or delayed occurrence of any findings after a 2-month recovery period. Toxicokinetic findings of the study are described in a separate manuscript (Dahms et al., 2016). In this study, the low dose of 150 mg/kg bw/day was selected based on the maximum expected human dose of DHA-EE 4 g/day. The 4 g/day dose corresponds to 120 mg/kg bw/day in dogs according to the allometric scaling conversion based on the body surface area (FDA, 2005). The high dose of 2000 mg/kg bw/day was selected based on a limit dose determined by ICH Harmonized Guideline M3(R2) (ICH, 2009) and results of the 8-week study. The intermediate dose 1000 mg/kg bw/day was intended to exhibit a potential gradient of effects.

2. Methods

The study was conducted at Charles River Laboratories Preclinical Services in Spencerville, Ohio, USA in compliance with the U.S. FDA Good Laboratory Practice (GLP) regulations. The design of this study was based on FDA Redbook 2000, General Guidelines for Designing and Conducting Toxicity Studies and ICH Harmonised Tripartite Guideline M3 (R2).

2.1. Animals

Twenty-two male and 22 female Beagle dogs were received from Marshall BioResources, North Rose, New York. They were 6–7 months of age at the time of randomization with body weights ranging from 6.1 to 7.7 kg for the males and 5.5–6.9 kg for the females. The animals were allowed to acclimate to the laboratory environment for 11 days prior to the first day of dosing (Day 1). Before randomization procedures (Day -3), the animals were weighed and examined in detail. Animals determined to be suitable as test subjects were randomly assigned to groups (3 animals/sex/group for main phase and 2 animals/sex/group for recovery phase) by a stratified randomization scheme designed to achieve similar group mean body weights. Prior to the main phase termination, recovery animals were randomly selected with no restrictions on randomization. Number and age of animals were selected in accordance with the FDA Redbook 2000 guidance for chronic dog studies (Chapter IV.C.5.b.). The guidance recommends to have at least 4 dogs per sex per dose group which should be no older than 6 months of age at the beginning of the study. The animals were housed individually in suspended stainless steel cages during acclimation and while on study. Housing and care were as specified in the USDA Animal Welfare Act (9 CFR, Parts 1, 2, and 3) and as described in the Guide for the Care and Use of Laboratory Animals (NRC, 1996). Room temperature and relative humidity were recorded continuously at 15-min intervals and the daily averages ranged from 66 to 72 °F (19–22 °C) and 45–63%, respectively. The 12-h light/dark cycle and 10 air changes per hour with 100% fresh air were used. Teklad Dog Diet 2025 from Harlan Laboratories was provided as a daily ration throughout the study. The diet contained 26.0% of protein and 10.5% of fat. The principal fatty acids in the diet were palmitic (1.9% of the diet), stearic (0.5%), oleic (3.4%), linoleic (2.8%) and α-linolenic acid (0.2%). The diet did not contain arachidonic acid. An approximate 300-g ration of feed was provided daily to each dog beginning on the day after receipt.

2.2. Experimental design

DHA-EE in corn oil vehicle and corn oil control were administered by once daily oral gavage on Days 1–273 according to the experimental design illustrated in the Table 1. Main study animals (3 per sex per group) were terminated on Day 274, and the recovery animals (2 per sex per group) – on Day 330.

2.3. Test articles

The vehicle/control article was corn oil. The test article DHA-EE (purity 900 mg/g, determined by a gas chromatography mass spectrometry) was administered neat to the high dose animals or diluted with the corn oil for other animals (refer to Table 1). The dose volume for each animal was 2.2 mL/kg bw/day and was based on the most recent body weight measurement. Dose formulation samples collected on Day 1, 78, 157, 239 and 267 were analyzed by a validated gas chromatography/flame ionization detector and under GLP for homogeneity and concentration verification, as well as for stability. Stability of dose formulations was established for frozen and refrigerated storage, as well as for ambient conditions. Concentration results were considered acceptable if the difference between the mean value found and the targeted concentration was within 15%. Homogeneity results were considered acceptable if a relative standard deviation (RSD) of the mean value at each sampling location was within 5%. All preparations met acceptance criteria. There was no DHA-EE detected in the control. DHA-EE and corn oil were stored frozen at −20 °C and were transferred to storage at 5 °C ± 3 °C no more than 7 days before first use. Test materials were placed at ambient temperature and stirred for a minimum of 1 h before dose administration.

2.4. Clinical observations

The animals were observed twice a day for general health/mortality and moribundity. Each animal was observed in detail weekly prior to being weighed. Food consumption was measured each day. The following examinations were performed once prior to in-life initiation, once during the last week of treatment (Week 39) and once during the last week of the recovery phase (Week 47): physical examinations by a staff veterinarian including overall condition and body temperatures; ophthalmologic examination by a board-certified veterinary ophthalmologist using a hand-held slit lamp and indirect ophthalmoscope preceded by dilation using short-acting mydriatic solutions instilled into each eye; electrocardiogram (ECG) tracings (Leads I, II, III, aV_R, aV_L, and aV_R collected but Lead II examined) by a board-certified veterinary cardiologist.