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Subchronic (13-week) oral toxicity study, preceded by an in utero exposure phase, with arachidonate-enriched triglyceride oil (SUNTGA40S) in rats

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Abstract

Polyunsaturated fatty acids (PUFAs), such as arachidonic acid (ARA) and docosahexaenoic acid (DHA) are natural constituents found in human milk, fish oil or egg yolk. Until recently, infant formulas, though providing the essential fatty acid precursors for these PUFAs, did not contain preformed ARA or DHA.

In this study the safety of SUNTGA40S as source of ARA, not only for use in infant formulas but also for nutritional products or food supplements, was evaluated in a subchronic study in Wistar rats, preceded by a 4-week pretreatment period of parental (F_0) rats and exposure of the F_0 dams throughout mating, gestation and lactation. SUNTGA40S was administered at dietary levels of 0.5%, 1.5% and 5% (wt/wt) adjusted with corn oil to 5.76% added fat. An additional group received 3.65% (wt/wt) SUNTGA40S in conjunction with 2.11% (wt/wt) high DHA Tuna oil, providing an ARA:DHA ratio of 2.7:1. High-fat and low-fat controls received basal diet with or without 5.76% corn-oil supplement.

The content, stability and homogeneous distribution of the test substances in the diet were confirmed under study conditions. The administration of SUNTGA40S, with or without DHA oil, did not affect health, growth, fertility or reproductive performance of the parental rats, nor pup characteristics (condition, weight gain, viability, number per litter or sex ratio). In the subchronic study with the offspring (F_1) rats, no significant differences were found in condition, neurobehavioural observations, ophthalmoscopy, growth, urinalysis or macroscopic and microscopic findings between the test groups and the low-fat or the high-fat controls. In males of the 5% SUNTGA40S and the SUNTGA40S/DHA group, red blood cell counts, haemoglobin concentration and packed cell volume were lower and reticulocytes were slightly higher than in the high-fat and low-fat control groups. Cholesterol, triglycerides and phospholipids in plasma were lower than in the high-fat controls in both sexes in the 5% SUNTGA40S and the SUNTGA40S/DHA group and (for triglycerides only) in the 1.5% SUNTGA group. Due to the administration of extra dietary fat, food intake and prothrombin time (males only) were lower and alkaline phosphatase activity was higher in all the high-fat groups, including the corn-oil controls, as compared to the low-fat controls. The weight of the spleen was higher in males of the 5% SUNTGA40S and the SUNTGA40S/DHA group compared to both the low-fat and the high-fat controls. The effects noted in this study at high dose levels of SUNTGA40S are consistent with previously reported physiological responses to dietary intake of high PUFA containing oils. The present results provide evidence that SUNTGA40S is a safe source of arachidonic acid. Except during lactation when the intake in dams doubled, 5% Suntga40S in the diet was equivalent to an overall intake of approximately 3 g/kg body weight/day in F_0 and F_1 animals.

Abbreviations: ARA, arachidonic acid; DHA, docosahexaenoic acid; PUFA, polyunsaturated fatty acid; MCV, mean corpuscular volume; MCHC, mean corpuscular haemoglobin concentration.

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1. Introduction

The human body can synthesize ARA and DHA through desaturation and elongation of the 18-carbon fatty acids linoleic acid (C18:2($n-6$)) and linolenic acid (C18:3($n-3$)). The human fetus and neonate initially obtains ARA and DHA by placental transfer and via human milk. In contrast to human milk, standard infant formulas contain only trace amounts of ARA and DHA.

Many advisory bodies (e.g. ESPGAN, 1991; the British Nutrition Foundation Task Force, 1992, SCF, 1993) and other scientists have suggested that infant formulas should contain the same amounts of DHA and ARA as human milk. For these reasons, there is an increasing interest in highly purified oils rich in ARA or DHA, because they can be mixed to provide a ratio of ARA/DHA similar to that of human milk while minimizing the exposure to other fatty acids.

Furthermore other arguments stress the need for fortifying human diet with ARA. There is an age-dependent decrease in the concentration of ARA in the hippocampus, and aged rats exhibit an impaired ability to sustain long-term potentiation (LTP) (Soderberg et al., 1991; McGahon et al., 1997). The age-dependent suppression of LTP is restored by chronic supplementation of ARA or γ-linolenic acid (McGahon et al., 1997).

Many safety studies of ARA enriched triglyceride obtained from *Mortierella alpina* have been reported (Streekstra, 1997; Hempenius et al., 1997, 2000). The objective of this study was to assess the safety of SUNTGA40S, a newly extracted oil from *M. alpina* and highly purified, in a subchronic study in F_1 rats, preceded by a 4-week pretreatment period of parental (F_0) rats and exposure of the F_0 dams throughout mating, gestation and lactation. This design was used in order to mimic the intended exposure of pregnant women and infants to the oil. SUNTGA40S was administered at dietary levels of 0.5%, 1.5% and 5% (wt/wt). Because feeding high levels of SUNTGA40S might result in an imbalance between $n-6$ and $n-3$ polyunsaturated fatty acids, SUNTGA40S was also administered at a level of 3.65% in conjunction with 2.11% high DHA Tuna oil. This combination group provided a total of ARA + DHA identical to the amount of ARA in the 5% SUNTGA40S group and a ratio ARA:DHA of 2.7:1, considering the ARA and DHA ratio of human milk reported in many papers.

Average ARA and DHA contents in total fatty acids and ARA/DHA ratio of breast milk are 0.1%, 0.3%, 0.3 for European and African women (Koletzko et al., 1992); 0.36%, 0.22%, 1.6 for German women (Kohn et al., 1994); 0.6%, 0.1%, 6.0 for American women (Putnam, 1982); 0.54%, 0.59%, 0.91 for women on a balanced meat and vegetable diet (Sanders et al., 1978); 0.72%, 0.23%, 3.1 for women consuming a vegan diet in United Kingdom (Sanders et al., 1978); and 0.35%, 1.46%, 0.23 for Japanese women (Tanaka et al., 1994), respectively.

2. Materials and methods

The study was conducted in accordance with the OECD Principles of Good Laboratory Practice (OECD, 1998b), and conformed to OECD Guidelines for the Testing of Chemicals 408 (OECD, 1998a) and EEC Directive 87/302/EEC (1988).

2.1. Materials

Arachidonate-enriched Triglyceride oil (SUNTGA40S), a clear yellow oil, lot number 01030351, produced by Suntory Limited, Osaka, Japan, was extracted from a biomass of submerged fermented *M. alpina* and refined by high purification processes. SUNTGA40S contained 41.5% arachidonic acid (ARA; C20:4($n-6$)), 0.1% eicosapentaenoic acid (EPA; C20:5($n-3$)) and no docosahexaenoic acid (DHA C22:6($n-3$)). It had a peroxide value of 0.42 meq/kg, low unsaponifiable matters not more than 1.0%.

DHA-containing oil (high DHA Tuna oil), a whitish cloudy oil, lot number 030121 was obtained from Nissui, Tokyo, Japan. High DHA Tuna oil contained 26.6% docosahexaenoic acid (C22:6($n-3$)), 7.1% eicosapentaenoic acid (C20:5($n-3$)) and 0.5% total tocopherols (peroxide value 0 meq/kg). Both test materials were stored in a freezer (<−18 °C) under nitrogen. The reference substance (corn oil), lot numbers L405366 and L405195 was obtained from Oliehoorn, Zwaag, The Netherlands and stored in a refrigerator (2–10 °C).

2.2. Animals and maintenance

The welfare of the animals was maintained in accordance with the general principles of the European Communities (Directive 86/609/EEC) and the Netherlands legislation (the Experiments on Animals Act 1997), governing the use of animals in toxicity experiments. Parental (F_0) male and female rats (75 males and 150 females), Wistar outbred (Crl:(WI)WU BR), were obtained from Charles River Deutschland, Sulzfeld, Germany. At the