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Two-generation reproductive and developmental toxicity assessment of dietary *N*-acetyl-L-aspartic acid in rats

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ABSTRACT

N-acetyl-L-aspartic acid (NAA) is a component of the mammalian central nervous system (CNS) that has also been identified in a number of foods. This paper reports the outcome of a reproductive toxicology study conducted with NAA in Sprague–Dawley® rats. NAA was added to diets at target doses of 100, 250 and 500 mg/kg of body weight/day and administered for two consecutive generations. A carrier control group was administered diet with no added NAA and a comparative control group was given aspartate (ASP), the constituent amino acid of NAA, at a target dose of 500 mg/kg of body weight/day. The study evaluated OECD 416 reproductive performance variables and additional segments to assess potential developmental effects, neurobehavioural and ophthalmologic function, and the concentrations of NAA or ASP in brain and plasma. No biologically significant differences were observed in any reproductive response variables, neurobehavioural tests, ophthalmologic examinations, body weights, feed consumption, or organ weights. Further, no test substance related mortalities or adverse clinical, neurohistopathologic or histopathologic findings were observed. Under the conditions of this study, the highest target dose of NAA, 500 mg/kg of body weight/day, represents the no-observed-adverse-effect-level (NOAEL) for reproductive and systemic toxicity, and neurotoxicity for Sprague–Dawley® rats.

1. Introduction

N-acetyl-L-aspartic acid (NAA) is the *N*-acetylated derivative of the amino acid L-aspartate (ASP) that is present at high concentrations in the central nervous system of humans and other animals (Tallan et al., 1956; Kirmani et al., 2002; Baslow, 2003). It is synthesized via enzymatic acetylation (cofactor = acetyl-CoA) of aspartate by *L-aspartate-N-acetyltransferase* (E.C. 2.3.1.17) in the mitochondria of neurons after which it is transferred to the cytosol (Truckenmiller et al., 1985; Demougeot et al., 2004). The *L-aspartate-N-acetyltransferase* enzyme is expressed exclusively in the neurons of the CNS. NAA is metabolized to acetate and aspartate in the CNS by *N-acetyl-L-aspartate aminohydrolase* (E.C. 3.5.1.15; also known as *aspartoacylase*) which is expressed in glial cells and oligodendrocytes but not in neurons or astrocytes (Demougeot et al., 2004; Baslow, 2000). This restricted distribution of the biosynthetic and metabolic enzymes accounts for the high concentrations of NAA within the neurons of the CNS and low concentrations outside of the neurons.

The CNS possesses a high capacity to synthesize and metabolize NAA because acetate liberated from enzymatic deacetylation of NAA is the primary source of free acetate for the biosynthesis of fatty acids which are used in the myelination of neurons of the mammalian CNS during postnatal development (D'Adamo et al., 1968; Patel and Clark, 1979; Chakraborty et al., 2001; Madhavarao et al., 2005; Namboodiri et al., 2006). The critical role of NAA in this process is exhibited in Canavan disease, a heritable metabolic disease caused by mutations in the *aspartoacylase* gene that results either in lack of expression or expression of a non-functional form of the enzyme (Kaul et al., 1993; Zeng et al., 2002; Hershfield et al., 2006). Accordingly, the concentration of NAA in the brains of persons with Canavan disease is higher than that of healthy individuals (Kumar et al., 2006). The clinical outcome of this disease includes mental retardation, lack of motor skills, muscle control, seizures, macrocephaly, optic atrophy, marked developmental delay, and premature death (Gambetti et al., 1969; Al-Dirbashi et

Abbreviations: ASP, aspartate; CNS, central nervous system; F1, first filial generation; F2, second filial generation; E.C., Enzyme Commission; EPA, Environmental Protection Agency; GLP, Good Laboratory Practice; LD, lactation day; NAA, *N*-acetyl-L-aspartic acid; NOAEL, No-observed-adverse-effect-level; OECD, Organization for Economic Co-operation and Development; P, parental; PND, postnatal day; SS, subset.

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al., 2007; Moffett et al., 2007). Histological changes including spongiform degeneration in brain white matter and global hypomyelination have been well documented (Kamoshita et al., 1968; Adachi et al., 1973).

Some investigators have hypothesised that the elevated concentration of NAA in the brains of persons with Canavan disease is responsible for the pathology (Baslow, 2000). Additional publications have reported adverse effects in laboratory animals following injection of NAA directly into the CNS (Akimitsu et al., 2000; Kitada et al., 2000). However, it is now widely believed that the clinical and histopathologic changes of Canavan disease are attributable to the inability to liberate acetate from NAA during development of the CNS (acetate deficiency hypothesis) rather than being directly attributable to elevated concentrations of NAA (Mehta and Namboodiri, 1995; Burlina et al., 1999; Kirmani et al., 2002; Madhavarao et al., 2005).

It was recently reported that NAA is present in a number of foods including vegetables, fruits, dairy, eggs, meat and grains (Hession et al., 2008) raising the question of whether dietary exposure to NAA could result in adverse effects. We previously reported no adverse effects in rats following dietary administration of NAA (Delaney et al., 2008 and Karaman et al., 2011) and no evidence of mutagenicity (Karaman et al., 2009) although it was acutely toxic following oral gavage at extremely high doses (i.e., 5000 mg/kg of body weight; Delaney, 2010). This paper reports the outcome of a two generation reproductive toxicity assessment of NAA in Sprague–Dawley® rats. An additional group of rats was administered 500 mg of ASP/kg of body weight/day as a comparative control to address the possibility of elevated dietary exposure to ASP because of the activity of endogenous acylases (Kaul et al., 1993; Giardina et al., 1999). The study was conducted according to OECD testing guideline 416 (2001) and under Good Laboratory Practice (GLP) compliance (EPA, 1989). Additional segments were incorporated to evaluate potential effects on development, neurobehavioural and ophthalmological function. The concentrations of NAA and ASP in brain and plasma were also evaluated as contradictory outcomes with regard to the reports of the ability of exogenously administered NAA to enter the brain (Berlinguet and Laliberte, 1966; Snichkin et al., 1977).

2. Materials and methods

The study was conducted at Charles River Laboratories Preclinical Services (CRL; Horsham, PA). A schematic presentation of the study design is shown in Fig. 1. F1 and F2 progeny were divided into eight subsets (SS) as described in Table 1 to study additional response variables that are not a requirement of OECD Guideline 416 (see also Supplemental Methods available on-line).

2.1. Test and control substances, doses and dietary formulations

The test substance (*N*-acetyl-L-aspartic acid; NAA; 100% pure) was obtained from the Sigma–Aldrich Co. (St. Louis, MO, Catalog No. 00920). The comparative control substance (L-aspartic acid; ASP) was obtained from VWR International, LLC (West Chester, Pennsylvania; Catalog No. AS125). Multiple vials of ASP from different lots were used in the study and the purity of the ASP in these lots ranged from 99.3% to 100.4% as reported in the manufacturer's certificates of analysis.

A commercially sourced rodent laboratory diet in meal form (Certified Rodent LabDiet® #5002, PMI® Nutritional International, St. Louis, MO) served as carrier. NAA was administered in the diet at three target doses; 100, 250 and 500 mg/kg

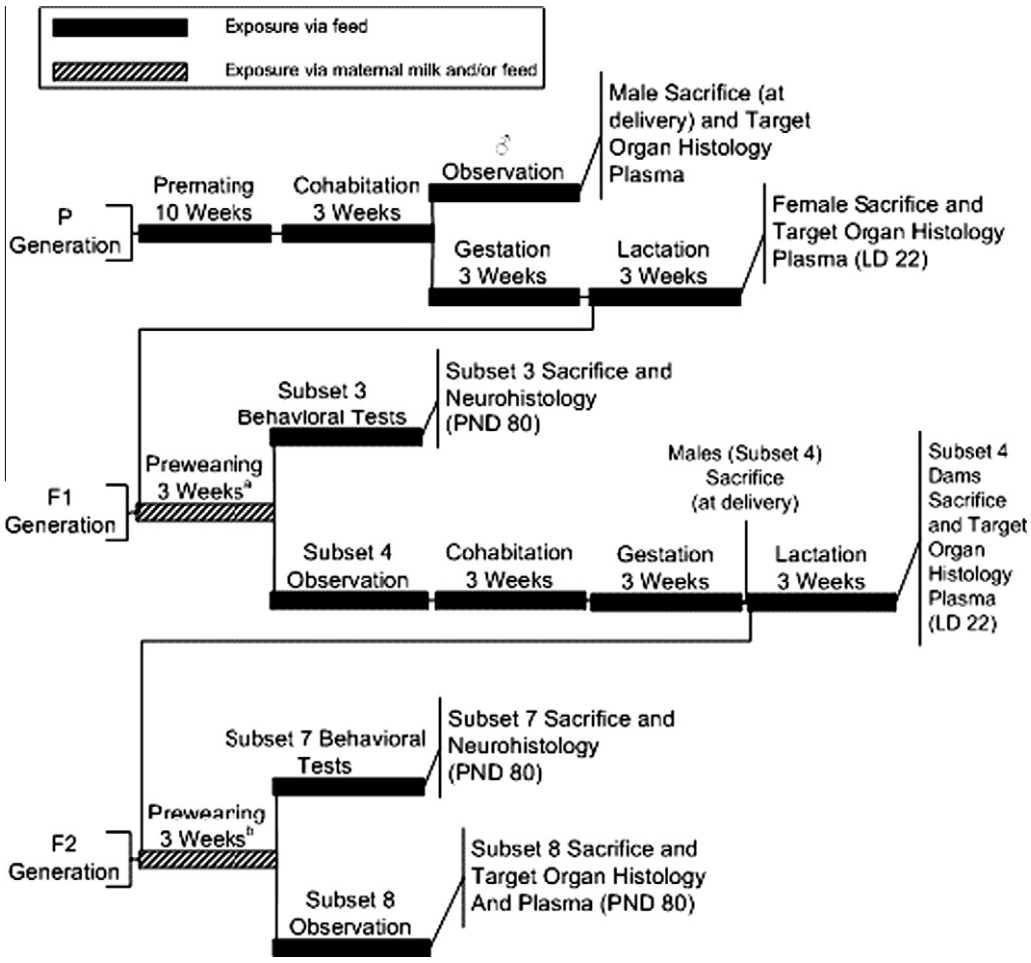

Fig. 1. Schematic illustration of study design. PND, Postnatal day; LD, Lactation day. Subsets 1, 2, 5 and 6 were culled and sacrificed at the time of weaning.