Establishment of the Safety in Use of Two Synthetic Human Milk, Nature-Identical, Oligosaccharides 2’-O-Fucosyllactose (2’FL) and Lacto-N-Neotetraose (LNNT) for Infant Formula

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Introduction:
In order to match the composition of human breast milk more closely, it is now possible to supplement commercial infant formula with synthesised oligosaccharides that are chemically identical to human milk oligosaccharides. There is currently no official guideline available specifically addressing the data required for safety assessment of such materials. The safety approach developed for two new human-identical milk oligosaccharides (HiMOS), 2’-O-Fucosyllactose (2’FL) and Lacto-N-neotetraose (LNnT) is presented here.

Methods:
This consisted first of chemical characterization to establish the structural equivalence with the natural endogenous molecule and to get insight on potential impurities resulting from processing. Pre-clinical in vitro and in vivo investigations were then conducted to establish the safety boundaries of the materials and to identify potential unexpected concerns. In vitro genotoxicity testing (bacterial reverse mutation, mouse lymphoma and micronucleus tests) was first conducted. Then, to establish the toxicological profile of the materials, 2’FL and LNnT were administered each separately via gavage in a model representative of the intended target population (a juvenile adapted sub-chronic 90 day rat study). Data from juvenile adapted 14 and 28 day rat studies were also available for LNnT. Fructooligosaccharide (FOS) currently approved for use in infant formulae was used as a reference control.

Results:
2’FL and LNnT were non-mutagenic in in vitro assays. Oral administration up to 5000 mg/kg bw/day of either 2’FL or LNnT to rats (starting to post-natal day 7) over 90 days was not associated with any adverse effects, based on clinical observations, body weight, feed consumption, clinical pathology, organ weights and histopathology findings. The 90-day study together with genotoxicity data was considered most relevant to identify potential toxicological effects resulting from the presence of process-related, uncharacterized impurities, whilst the shorter studies better addressed potential sub-acute gastrointestinal tolerance aspects. These later effects may be missed in longer-term studies because of reversibility resulting from lower susceptibility in adulthood. In addition, pre-clinical data provided insight into the upper bound gastrointestinal tolerance of the ingredient in order to establish a margin of exposure compared to the anticipated human intake. These findings allowed further investigation in clinical trials.

Discussion:
These findings in the juvenile rat have supported the safety of LNnT and 2’FL for use in infant foods and have supported authority decisions for such applications and target populations. Both 2’FL and LNnT have been generally recognized as safe (GRAS) by the US FDA. In Europe, the NDA Panel at EFSA concluded that LNnT is safe for infants (≤ one year of age) when added to infant and follow-on formulae, and for young children (> one year old) when added to follow-on and young-child formulae in combination with 2’-FL, at concentrations up to 0.6 g/L of LNnT and up to 1.2 g/L of 2’-FL, at a ratio of 1:2 in the reconstituted formulae.

Keywords: Safety, Human Milk Oligosaccharides, Lacto-N-Neotetraose, 2’-O-Fucosyllactose, Preclinical studies, Probiotics
Citation: