Supporting document 4

Summary of Conclusions: A1155 Independent Expert Advisory Group

Food Standards Australia New Zealand established the A1155 Independent Expert Advisory Group (IEAG) to provide expert advice, opinion and information relevant to the Application A1155 review response. In particular, FSANZ sought the IEAG's advice on its approach to the assessment of the benefits of 2'FL and LNnT used in infant formula products and formulated supplementary foods for young children; the strength and adequacy of the evidence base; and the appropriateness of the conclusions of the assessment. This document summarises the advice and key conclusions of the IEAG discussions. This has been taken into account in FSANZ's benefit assessment as part of the review report, as appropriate.

Bifidogenic effect

For the assessment for infants the IEAG were asked to consider FSANZ's approach to the assessment of a bifidogenic effect for 2'-FL and LNnT. Also to consider whether normal development for infants includes changes to gastrointestinal microbiota; the relationship between levels of 2'-FL and LNnT in human milk and levels of bifidobacteria; links between bifidobacteria in the infant intestinal microbiome and positive health outcomes as well as the evidence for the effect of supplementation of infant diets with 2'-FL and LNnT on levels of bifidobacteria and health outcomes.

The IEAG agreed that FSANZ's approach - comparing the microbiome compositions of breastfed vs formula fed infants, with regard to levels of bifidobacteria, their species composition and oligosaccharide intake is appropriate.

Normal human development requires a microbial community, and this is sustained by components of the breastmilk that is targeted at microbes, and in healthy infants and young children undergoing natural development the microbial species include members of the genus *Bifidobacterium*, and in breast fed infants these are typically predominant. There are differences between human milk and infant formula. It is accepted that there is a strong association between breast milk and bifidobacterial abundance in infants. Given that breast milk and formula differ greatly in oligosaccharide quantity and quality it is reasonable to link human milk oligosaccharides (HMOs) to bifidobacterial abundance, given the ability of the bacteria to utilise HMOs for growth (as demonstrated in vitro and in vivo). While there is evidence of a relationship, with 2'-FL and LNnT in human milk and bifidobacteria, the studies have many limitations and the relationship is not strong. But if you consider ability of some bifidobacteria to use 2'-FL for growth the potential is there, but it is not yet possible to see that potential fulfilled in trials in children. The evidence supports that the presence of 2'-FL/LNnT can stimulate growth of bifidobacteria but that there is not a straight line correlation.

It has been accepted in paediatrics for decades that infants who receive breastmilk have less GI upsets, less ear and respiratory infections; while bifidobacteria may have an important role there are many other factors that may impact upon health outcomes. Consequently, it is not possible to determine a linear effect from the presence of a microbial nutrient in human milk and a specific health outcome for the infant. We should not be too tied by a specific health outcome, but should focus on a specific mechanism by which the microbiome is modified to one recognised as both safe and associated with desirable health outcomes . There are many specific mechanisms which influence health outcomes.

There is a bifidogenic effect resulting from of supplementation of infant diets with 2'-FL and LNnT - evidence for this is robust. However, there is limited evidence to specifically link the

bifidogenic effect to a positive health outcome. The evidence doesn't show a mechanism for this. It shows that the 2'-FL has a positive outcome, but the sample size is small. It's too simplistic to link total numbers or relative abundance of bifidobacteria to health outcomes. A number of bifidobacteria species inhabit the infant gut and each has different ability to grow on HMOs of different types. It is more a question of quantity and quality.

The IEAG noted that the term 'Dose repose' is not appropriate in this context as it is based on pharmacodynamics effect. Oligosaccharides don't work that way, for example the mechanism is microbial growth on the substrate but the microbial response is controlled by whatever nutrient is limiting for that bacterial species. Therefore, the dose of the prebiotic and HMO is not necessarily tied to the response.

Improvement of infant formula is important because many infants do receive some infant formula and deserve to have the best product available. For young children there is evidence to support the stimulation of bifidobacteria but you won't see it to the same effect as in infants and it is not as relevant for toddler milks as it is supplementary nutrition.

Pathogen binding inhibition effect

The EAG noted the approach outlined by FSANZ for this assessment and agreed it is entirely appropriate. The EAG agreed with the consideration of the evaluation of epidemiologic data relating to C. jejuni infection and breast feeding relative to formula feeding, breast milk and maternal secretor status, in vitro binding and inhibition assays, mouse model of infection/protection by 2'-FL showing a clear dose-response.

The IEAG agreed that, as outlined by FSANZ, 2'-FL binds to the pathogen surface, which inhibits the binding of the pathogen to receptors on the intestinal mucosa, thus preventing invasion. Worth noting that it is not clear if the pathogen is solely dependent on that binding interaction to the intestinal mucosa for infection to occur.

Based on the in vitro studies and the mouse model study there is a 'dose response effect' in relation to the competitive inhibition of binding of the pathogen to its receptor. However, this can't be extrapolated to a 'dose response' effect on reducing infection in infants or children, because those types of studies can't be done in humans.