**ISAPP response to IAC questions**
Posed at the ISAPP IAC meeting August 3, 2003

**Immune biomarkers that could be easily understood by consumers and healthcare providers**
The group agreed that at this stage there are no 'clinically substantiated and widely accepted' immunological biomarkers. Although modulation of the host immune function is reported to be one of the most common health benefits of probiotics, little is known about the relationship between immunoactivity and health improvement. The cellular and molecular mechanisms by which probiotics influence the functioning of the immune system also remain poorly understood. Thus, a lot more work needs to be done before a recommendation on such immunological biomarkers could be made. Properly designed clinical studies that measure health outcomes as well as indices of immune function (in the same subjects) are required to validate potential biomarkers.

**Strategies to advance science behind benefits of improved flora:**
A multi-disciplinary approach is the key (microbiologists, dieticians, nutritionists, clinicians, immunologists etc.) to advancing our understanding of the value of probiotic- or prebiotic-induced alterations in gut flora. Research should be hypothesis driven and underpinned through sound mechanism-based explanations of effect. For tracking changes in the gut flora, a molecular approach is critical, with culture independent methodologies becoming more widespread. Peer reviewed publications are essential and the results gained should be repeatable in other laboratories. Human studies are preferred but these can be backed up by *in vitro* or animal work to confirm or test mechanisms. Good biomarkers are required to assess fermentation, enzyme profiles, impact on health, cognitive function etc. These include immune markers, organic acids and other metabolites, shifts in the flora, clinical outcome, wellbeing markers, genome expression and microbial activity indicators. Models should be validated as reliable. Some examples of research approaches that may shed some light on the question include:

- Gut flora-reconstituted animal models that are devoid of specific test species
- Correlative studies of the gut flora of diseased vs. healthy humans

To communicate the message on the value of gut flora changes, the health care profession and reliable media sources were seen as important. ISAPP clearly could play a major role in harnessing skills and expertise. Research output should combine applied and fundamental science and therefore is likely to depend on different funding sources depending on the issue being questioned.

**Minimum effective dose for different applications:**
Different levels are likely required for different age groups, depending on the starting population of native beneficial bacteria. For example, if you begin with a low probiotic

level (such as often occurs in the elderly), then smaller changes in the flora levels may be more relevant than if the lactic flora is already high. Generally, at least a 0.5 log increase in response to prebiotics is suggested as evidence of an effect, but more likely a one log increase is required. Probiotic products should deliver viable probiotics at a level which has been documented to have a beneficial physiological effect. In general, products delivering less than $10^9$ per day have not been effective, except perhaps in effecting minor alterations on fecal microflora. Furthermore, there does not seem to be an upper limit to this, and in fact a published meta-analysis showed increased effectiveness with increased daily dose up to $10^{11}$/day. The relevant factor, however, is the existence of scientific documentation to justify formulation levels. This is not the case for prebiotics where too high a dose may cause side effects such as gas distension. Different pro-and prebiotics are likely to be relevant for different populations. New advances in next generation synthesis and unraveling of genomic effects can help to inform product choice.

**D, L -lactic acid:**
The Group agreed with the position paper circulated by Eamonn Connelly (copy may be requested from Eamonn at ec@biogaia.se). Essentially, authorities have generated some concern over lactic acidosis. However, lactate will disappear very quickly in the gut as it is the preferred electron sink product for the flora. Without this occurring, the anaerobic fermentation would be compromised. Any levels of lactate generated by product intake should therefore not have any negative biological impact. ISAPP can produce a position paper on this if Board of Directors agrees.

**Human origin of probiotic strains**:
The Group was asked its opinion if human origin was an important selection criterion for probiotics for human use. Several factors were considered. (1) The accepted definition of probiotic (FAO, 2001) does not stipulate any natural habitat for strains used as human probiotic. (2) There are many examples of probiotic strains from species not recognized as normal inhabitants of the human for which physiological benefits for humans have been documented. (3) Criteria do not exist to define use of the term ‘human origin’. Often, isolation from human feces is considered adequate ‘proof’ of human origin. However, it is clear that although many strains may survive intestinal transit, this does not imply that they can adhere or colonize or possess traits which predispose them to a more intimate association with humans. The term ‘human origin’ implies more than ‘survivability through the human GI system’, but in practice the term may be used without scientific evidence of more. Taken together, it seems that the property of ‘human origin’, especially as commonly used, is not a relevant criterion for probiotic strains for human use.

**Are undefined products probiotics?**
This question was raised with regard to traditional products (fermented foods, kefirs, etc.) prepared with undefined blends of many different genera, species and strains of bacteria and/or yeast. Although some scientific literature supporting the probiotic nature of some strains of the species contained in these products may exist, the microbes used in these products have not been isolated, characterized or defined. Although it is clear that health

benefits may result from consumption of these products, it is difficult to imagine being able to subject such undefined products to rigorous scientific testing for efficacy. As such, the health effects of these products cannot be substantiated and therefore cannot be termed 'probiotic'. A term such as 'functional food' would be more appropriate for these products.

**Health benefits of live vs. killed or dead probiotic cells:**
The definition of probiotic stipulates that the microbes administered be alive. Even though dead cells may mediate physiological benefits, albeit more moderate ones, they are not probiotics, and as such do not fall into the purview of ISAPP. Few studies specifically address the contribution of killed cells to any observed health benefit. Conducting such studies would contribute to the understanding of mechanisms of action of probiotics. Such comparative studies should be conducted.

**Definition criteria for prebiotics:**
The group felt that the following definition of prebiotic is fundamentally sound: A non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, that can improve the host health (Gibson, G.R. and Roberfroid, M.B. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J. Nutr. 1995; 125:1401-1412). This definition is based on knowledge of the number and identities of bacteria considered as desirable. Imminent research will certainly expand the understanding of this basic situation. It was also recognized that generalizing about the value of certain bacterial genera is neither prudent nor justifiable, as certain members of some genera (notably *Bacteriodes*) may be harmful or not, depending very much on which species is being considered. This is due to practical limitations in speciation of bacteria in complex environments and will improve in the future.

The group also felt that researchers and industry should respect the definition and not use the term prebiotic as equivalent to dietary fiber.

**Potential health benefits of prebiotics and how these should be communicated?**
The group felt that most of the health-related effects are the same as those proposed for probiotics although the weight of evidence for health benefits of prebiotics in humans is not as great and needs to be improved.

There may, however be health-positive outcomes of prebiotic intake that do not rely on bacterial fermentation - examples include stimulation of apoptosis in cancer cells and effects on blood lipids. The evidence for these effects is, however, at an early stage with little data coming from human studies.

**What non-digestible oligosaccharides are proven prebiotics?**
The group agreed that in order to be classed as a prebiotic, a carbohydrate had to be seen to have a prebiotic effect in at least one well-designed human trial with the microbial analysis being performed using culture-independent molecular techniques. Based on this criterion, the only proven prebiotics are fructo-oligosaccharides (whether derived from

sucrose or inulin), inulin, galacto-oligosaccharides and lactulose. There are a range of prebiotics on the Japanese market that have been studied in humans although the studies have sometimes involved small numbers of volunteers and the microbiology has rarely been carried out using molecular techniques. There are still more candidate molecules not yet tested in humans.

**Degree of evidence needed for claims** and **Product labelling for content and claims**
These topics were the main focus of the Weight of Evidence group at the 2003 ISAPP meeting and will be addressed adequately here. Thorough conclusions will be prepared by this group and circulated in a document to be published with the conclusions of the other groups.