Microbiological Risk Assessment Review of B. cereus

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October 4th, 2012

Manufacturer’s Responsibility
• Ensure that products are properly preserved
• Are safe and free from harmful bacteria during manufacturing
• Continues to remain free from contamination
• Comply with all internal QC specifications
• Comply with all local and federal governmental regulations

Tools for the Manufacturer and the Microbiologist
• Preservative Efficacy Test
• Aerobic Plate Count
• Clinical Use Study
• GMP Guidelines
• Protective Packaging
• Protective Storage and Filling Operations
• Microbial Risk Assessment (MRA)
Product versus Microbiological Risk Assessment

- **Product Risk Assessment** – is the ability of a product to support microbial growth and the factors used to determine that risk
- **Microbial Risk Assessment** consists of the identification of hazards and the evaluation of risks associated with exposure to those hazards
- **Hazard** is defined as an agent capable of causing an adverse reaction on the exposed individual
  - *Hazard could be either chemical or biological related*

Microbial Risk versus Product Risk Assessment

<table>
<thead>
<tr>
<th>Microbial Risk</th>
<th>Product Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host susceptibility</td>
<td>Type of Formulation</td>
</tr>
<tr>
<td>Microbial Growth</td>
<td>Preservative Efficacy</td>
</tr>
<tr>
<td>Health Endpoints</td>
<td>pH</td>
</tr>
<tr>
<td>Ability to adhere</td>
<td>Water activity</td>
</tr>
<tr>
<td>Ability to reproduce</td>
<td>Area of application</td>
</tr>
<tr>
<td>Single Exposure Outcome</td>
<td>Type of Packaging</td>
</tr>
<tr>
<td>Route of Exposure</td>
<td>Temperature of manufacturing</td>
</tr>
<tr>
<td></td>
<td>Temperature of filling</td>
</tr>
<tr>
<td></td>
<td>Water content</td>
</tr>
</tbody>
</table>

Recent Microbiological Methods, Standards and Guidelines Related to Product Risk Assessment

- **PCPC**
  - Method for the Preservation of Atypical Products
  - Microbiological Risk Factor Assessment of Atypical Cosmetic Products
- **ISO**
  - ISO 11930 - Evaluation of the Antimicrobial Protection of a Cosmetic Product
Objective of Today’s Presentation: Microbial Risk Assessment

- Present three different case studies related to *Bacillus species*
- Show how in a preserved cosmetic matrix, that these organisms should not be viewed as objectionable
- The importance of a Risk Assessment when determining potential risk of a cosmetic product.

3 Case Studies: Risk Assessment Review of B. cereus

- Grain workers in Britain
- Infant formula in Australia
- Imported Cosmetics in America

The HSE Grain Dust Study workers' exposure to Grain Dust Contaminants, Immunological and Clinical Response - 2007

- Objective of study:
  - To assess the exposure of grain workers in the UK to inhalable grain dust
- Number of subjects
  - 321 workers were exposed to grain dust from 1990 to 2003.
- Findings
  - Grain workers were frequently found to be exposed to more than 1 million bacteria and fungi per m³ air.
  - Levels of airborne endotoxin of over 10,000 EU/m³ were recorded at all but one workplace visited and personal exposures reached over 600 EU/m³ at every workplace.
Bacterial Counts

• Summary of Aerobic Bacterial Levels measured at farms and docks

<table>
<thead>
<tr>
<th>Farms</th>
<th>Bacteria</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 1</td>
<td>5.8 x 10^4 - 1.05 x 10^9</td>
<td>1.8 x 10^3 - 10^7</td>
</tr>
<tr>
<td>Phase 2</td>
<td>1.09 x 10^3 - 1.6 x 10^6</td>
<td>400 - 2.9 x 10^7</td>
</tr>
<tr>
<td>Phase 1</td>
<td>8.1 x 10^3 - 1.4 x 10^11</td>
<td>2.5 x 10^4 - 10^9</td>
</tr>
<tr>
<td>Phase 2</td>
<td>6.6 x 10^3 - 3.1 x 10^8</td>
<td>193 - 8.1 x 10^6</td>
</tr>
</tbody>
</table>

Bacterial taxonomy isolated from airborne grain dust

<table>
<thead>
<tr>
<th>Gram Negative Bacteria</th>
<th>Gram Positive Rods</th>
<th>Molds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agrobacterium</td>
<td>Bacillus licheniformis</td>
<td>Alterneria</td>
</tr>
<tr>
<td>Enterobacter agglomerans</td>
<td>Bacillus subtilis</td>
<td>Aspergillus</td>
</tr>
<tr>
<td>Pseudomonas corrugata</td>
<td>Microbacterium species</td>
<td>Cladosporium</td>
</tr>
<tr>
<td>Pseudomonas diminuta</td>
<td></td>
<td>Eurotium</td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>Coeci</td>
<td>Penicillium</td>
</tr>
<tr>
<td>Pseudomonas glycinelines</td>
<td>Micrococcus species</td>
<td>Yeasts</td>
</tr>
<tr>
<td>Pseudomonas maltophilia</td>
<td>Staphylococcus cohnii</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas marginalis</td>
<td>Staphylococcus epidermidis</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas testeroni</td>
<td>Staphylococcus xylosus</td>
<td></td>
</tr>
<tr>
<td>Xanthomonas oryzae</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Conclusion - Grain Workers Study

• There were no reported cases of traumatic eye infections even though the bacterial and fungal counts exceeded 1 x 10^9 c.f.u per gram
• Long term exposure to high endotoxin, bacterial and particle levels, did not have an effect on lung function and did not appear to cause chronic lung damage.
• Some subjects did have both symptoms of occupational asthma and sensitization to work related allergens.
Final Assessment Report
Bacillus Cereus Limits in Infant Formula

Australian – New Zealand Food Standards (FSANZ)
February 18, 2004

Objective
• To amend the limit of *B. cereus* in infant formula.
• The current standard is too restrictive and cannot be complied with consistently under good manufacturing practices.
• The objective is to raise the spec from <10 cfu per gram to <100 cfu per gram

Overview
• *B. cereus* spores can occur in milk at very low levels.
• There are seasonal variation in these counts.
• Processing of milk into powder for infant use will not eliminate spores.
• Illness from *B. cereus* spores can produces mild illness such as vomiting and diarrhea from foods at the >10⁶ cfu per gram range.
Overview

- Infant formula can be the sole source of nutrition for infants and therefore frequency of consumption is very high.
- Infant formula is considered a high risk food due to the greater susceptibility of infants.
- Infectious dose of *B. cereus* for infants is of concern because their immune system are not fully developed and are susceptible to bacterial infections.
- It is estimated that the rate of illness in the USA is 0.1 cases per 10,000,000 per year due to *B. cereus*.

International Microbiological Specifications for *B. cereus* in infant foods

- U.S. FDA: NMT 1000 cfu per gram
- Canada: <100 cfu per gram
- Netherlands: <100 cfu per gram
- Portugal: <100 cfu per gram
- Switzerland: <1000 cfu per gram

Survey of B. Cereus Contamination in Foods

- 52% of 1546 food ingredients
- 44% of 1912 creams and deserts
- 52% of 431 meat and vegetable products
- Up to 48% of dairy products
- 50% of UHT Milk
- Conclusion: Bacillus species is widespread in food products

1. Nygren 1962
2. ICMSF 1996
Risk Assessment Study concluded:

- Powdered infant formula containing 100 cfu per gram of *B. cereus* and reconstituted with 25 °C (77 °F) water and stored for 24 hrs would not expose infants to an infectious dose.
- Formula reconstituted with cooled boiled water and stored for 24 hours at 10 °C (50 °F) would not expose infants to an infectious dose.
- Formula made with powder containing 1000 cfu per gram of *B. cereus* and stored at 10 °C do pose a risk to infants.

Conclusion - Infant Formula Study

- That levels between 10 c.f.u – 100 c.f.u of *Bacillus species* was not a risk to infants when prepared and stored properly.

Case Study 3

Imported Cosmetics

- If you had a lipstick or a eye-shadow containing <10 CFU per gram of *B. cereus* ......
  - You would be delivering 0.01 – 0.05 grams of product per application
  - You would be delivering 10° cfu per gram of a bacteria per application of a preserved anhydrous product
  - This is equivalent to 0.001 cfu per gram of product
  - To an area of the body which contains a bacterial load that could be 1000x (lips) more than what is being delivered by the product.
Definition of Risk

- The degree of risk depends on the ability of the product to support the growth of the bacteria combined with the probability of those organisms to cause harm to the user.

Risk Analysis Review

- In **Case 1** you had a high bacterial load ($10^9$) in the eye area with no adverse reactions reported and no long term adverse reactions observed.

- In **Case 2** you had a product that if not stored correctly, being given to an immune compromised population, containing levels of bacteria that could possibly range from $<10^2$ - $<10^5$ cfu per gram and this found this to be acceptable.

- In **Case 3** you have a preserved or anhydrous hot pour product which is hostile to microbial growth containing levels of $<10$ cfu per gram of an organism(s) to an area of the body that may contain bacterial levels that could range from $10$ – $1000$ times higher than what is being delivered by the product.

- **So where is the risk?** As compared to the other two scenarios if a risk assessment was performed it would clearly shown a minimal chance of creating an adverse reaction.
Conclusion

- Data presented showed that in a non-cosmetic application, *B. cereus* not to be harmful even at levels 100x higher than limits used for cosmetic products.
- Cosmetics are not manufactured under sterile conditions
- Low levels of Gram positive bacteria have been accepted and there are no reported cases of adverse reactions

Conclusion

- When a proper risk assessment is performed, it can be a valuable tool in determining the susceptibility of a cosmetic formulation and ....
- Determining the potential risk to the consumer if not properly formulated, manufactured and packaged

References

- Final Assessment Report Application A454 - Bacillus Cereus Limits in Infant Formula - Food Standards Australia New Zealand February 18, 2004
- The HSE Grain Dust Study – Workers exposure to grain dust contaminants, immunological and clinical response – RR 540 Prepared by the Health and Safety Laboratory for the Health and Safety Executive - 2007