

## Study of microbial contamination in animal feed production units

This document provides a summary of a study financed by TECALIMAN, the ACTIA and the PAYS DE LOIRE REGION, all of the results of which were published in i'Doc\_S7 of July 1996.

### 1. Objectives

This involves identifying the risks of microbial contamination associated with the procedures and manufacturing equipment for compound feed. From a microbiological perspective, this study focuses on identifying four types of micro-organisms chosen by the industry: Salmonella, Enterobacteria, *Clostridium perfringens* and mycoflora.

### 2. Equipment and method

The study focuses on the first 7 phases of the HACCP method (See i'Tec\_S2). HACCP studies were conducted in three plants chosen by the profession between 1992 and 1994.

HACCP studies involve identifying factors, with the normal use of processes and in the event of foreseeable errors, that are likely to have an effect on the presence and/or growth of micro-organisms in plants. Measurement campaigns are carried out to clarify the risks. These campaigns include microbiological analyses and physical measurements (moisture, water activity, temperature). The latter must make it possible to assess whether the conditions necessary for micro-organisms to grow exist on the industrial sites in question.

### 3. Results and discussion

The data collected during the course of measurement campaigns, as well as the examination of process flow diagrams and plant practices, enables three decreasing levels of feed contamination risk to be determined.

#### 3.1. Level 1 risk

This **level** equates to the contamination of finished products by raw materials, product leaks or recycled feeds. Although the heat treatment applied does not enable it to be reduced, this initial contamination clearly represents the greatest risk of feed contamination. What's more, these components are the main contaminants of circuits causing the next level of risks.

#### 3.1.1. Raw materials

The raw materials used in animal feed carry significant quantities of germs. Although studying this source of contamination does not fall within the remit of this program, it is nevertheless partly addressed.

Consequently, testing for Salmonella focused on certain raw materials that are feed ingredients, the production cycle of which was monitored in full, and only where they were detected in the product at the mixer outlet. In eleven tests, this only occurred on one occasion, which is why only 7 raw materials form the subject of Salmonella testing. On this occasion, it is detected in two raw materials (a bran and a sunflower press cake) as well as in a recycled feed.

Enterobacteria testing was a little more extensive faced with a limited quantity of existing data: 8 samples on receipt and 29 after grinding.

The results reveal that rates of 100,000 Enterobacteria / g are quite common (7 cases out of 37, i.e. 19 % of samples). The most contaminated products are, in descending order: wheat, bran and barley, press cakes and peas.

*Clostridium* is not tested for in raw materials.

#### 3.1.2. Product leaks

Their fungal contamination remains low and represents storage mycoflora. In three cases out of four, these products contain a high level of Enterobacteria. It is possible to find Salmonella and *Clostridium perfringens* here too.

#### 3.1.3. Recycled feeds

Their levels of contamination with fungal flora, Enterobacteria and *Clostridium* are broadly less than those of raw materials and the water activity of these products does not exceed 0.70, which does not favour the growth of bacteria. However, it should be noted that three samples, originating from a plant, have Salmonella. The small number of cases examined do not provide conclusive evidence as regards the source of this contamination.

## 3.2. Level 2 risk

This level of risk applies to products contaminated by deposits when passing through the circuits. This risk is present if the deposits have a high microbial population. This may be due to:

- concentration phenomena associated with the manufacturing process (e.g.: aspiration of the smallest particles, i.e. vegetative cells, spores and contaminated dust in the air filters)
- ecological conditions favouring the growth of micro-organisms (Aw, temperature, duration).

### 3.2.1. Reception pit intake filters

It is possible to find very large Enterobacteria populations, greater than those that could be caused by raw materials, in these filters. It is also possible to find Salmonella here and storage fungal flora are present in large quantities. In certain cases, the presence of a significant population of *Clostridium perfringens* is observed.

### 3.2.2. Filters present along the route of unground raw materials

These filters have significant Enterobacteria, Salmonella, Clostridium and mould contamination.

### 3.2.3. Foot of the raw material elevator

Deposits present at this location point to a potential stagnation and growth site. Although this potential is contradicted by water activity measurements, a number of facts confirm it:

- large quantities of Enterobacteria
- the detection of *Scopulariopsis candida* and *Mucorales* in the two cases of fungal analysis (species that are characteristic of elevated water conditions).
- the presence of Salmonella in one sample and that of *Clostridium perfringens* in the four samples.

### 3.2.4. Grinder intake filters

These filters collect the dust generated by all ground raw materials. Two cases are examined.

#### 3.2.4.1 Externally vented filters

Contamination with Enterobacteria is reasonable. Fungal flora is at the right level. It is dominated by *Aspergillus glaucus*, which provides evidence of fairly low water activity, which is confirmed by measurements. The presence of Salmonella in three cases out of five is concerning as it suggests that these filters trap these germs. However, the prevailing conditions in this location suggest that Salmonella does not grow there or grows only sparingly depending on the opportunities.

#### 3.2.4.2 Filters vented into the plant

The filters in question were subject to variations in atmospheric conditions, which encouraged very strong fungal growth inside them.

## 3.2.5. Dosing cells

The deposits observed are subject to strong fungal growth, but their size varies depending on the raw materials. This growth may lead to a dispersal of mould spores from the tops of cells.

### 3.2.6. Mixer

All the samples taken from the mixers have water activity of between 0.72 and 0.79. This level of water activity may encourage the growth of mould at an ambient temperature, provided the residence time of these deposits in the mixer is sufficient. Consequently, the results of sampling carried out in an uncleaned mixer reveal a significant presence of Enterobacteria and mould requiring elevated water conditions (*Mucor*, *Scopulariopsis*), while sampling carried out in a cleaned mixer reveals far smaller growth of these populations, despite the same levels of water activity. Nevertheless, in both cases, the populations detected are of an acceptable level.

### 3.2.7. Molasser

The inside of this equipment does not reveal any significant deposits.

### 3.2.8. Foot of the meal mixture elevator

The observations and microbiological analyses carried out reveal that the deposits present on these elevator feet are:

- made up of mixtures of meals and molasses.
- the location for quite significant fermentation dominated, first and foremost, by yeasts and, secondly, by non-pathogenic moulds taking advantage of very damp environments (*Mucor* and *Scopulariopsis*), with Enterobacteria and other moulds seemingly excluded from this location.

Before becoming widespread, this observation requires verification at other sites.

### 3.2.9. Conditioner

The time elapsing between production being stopped and samples being taken appears to have an effect:

- Despite the presence of a significant deposit, a level of moisture and a temperature that are conducive to rapid microbial growth, no contamination is detected in the sample taken within the hour after production is stopped.
- After a stoppage of 4 to 36 hours without cleaning, significant growth is recorded in deposits that are often very moist. As a result, the flora present is frequently dominated by an opportunist species or strain: Enterobacteria, *Scopulariopsis candida*, *Aspergillus fumigatus*.

### 3.2.10. Press door

The results of two samples taken from the centre of press doors, after prolonged stoppages, reveal massive growth dominated by one or two species.

The moisture content of deposits sampled at the edges of doors is high. Nevertheless, in this case, these deposits have a low level of microflora, even after a lengthy period of stagnation (4 to 36 hours).

### 3.2.11. Coolers

This point in the process flow diagram can be viewed as a high risk point. In effect, it is a location within which condensation and numerous deposits of moist products occur, where the air circulation is very strong and where the temperature is higher than the ambient temperature. All these conditions are favourable for growth. To investigate this hypothesis, numerous samples of deposits are taken: 8 from countercurrent vertical coolers and 2 from horizontal coolers.

In the majority of cases, the results confirm the presence of significant microflora. It is often dominated by yeasts and moulds, among which it is possible to find toxinogenic germs (*Aspergillus spp.*). In certain cases, very high Enterobacteria contamination is detected, as well as the presence of Salmonella. These are frequently associated with the existence of very moist deposits.

The presence of *Clostridium perfringens* is detected in the two horizontal coolers used on a site contaminated by this species. However, it is not possible to attribute this presence to a single type of cooler, as they have virtually not been identified in the vertical coolers. An examination of samples taken in the immediate vicinity of horizontal coolers reveals the presence of *Clostridium* on the site. Therefore, this presence is interpreted as not being the result of growth, but of airborne spores conveyed by the intakes inside the plant.

### 3.2.12. Dust extraction systems

In the plants in question, two techniques are used to extract the dust contained in the air used during the course of drying-cooling pellets. The fine dust extracted is generally reintroduced into the manufacturing circuit at the cooler outlet or at the conditioner intake.

#### 3.2.12.1 Filters

Samples are taken simultaneously from the cooler and the filters. Contamination of these filters is extremely variable. Certain filters are carriers of deposits that are not very moist but are heavily colonised by mould. Others have almost no deposits but are carriers of Salmonella. Certain parallels can be noted between the moulds detected on the filters and those present in the coolers.

#### 3.2.12.2 Cyclo filters

The three samples of deposits taken all have extremely high levels of Enterobacteria and one of them is a carrier of Salmonella. In this case, bacterial contamination is combined with abundant fungal flora dominated by hydrophilic species. The significant moisture of these deposits is the cause of water activity that is higher than that observed in the majority of other locations.

### 3.2.13. Crumbler

The four samples taken lead us to believe that crumblers do not pose a significant danger of product contamination: a low level of deposits, a low Enterobacteria and mould population as well as a low level of water activity.

### 3.2.14. Coater

Of the four samples of deposits taken from this kind of equipment, the Enterobacteria count reveals that a single sample is heavily contaminated by these species. The results of this last sample can be explained, in part, by a high level of water activity. Mycoflora is at a similar level to that of raw materials.

### 3.2.15. Conveying finished products

Minor deposits are observed on the feet of finished product elevators and in a finished product conveyor/carrier located outside. They show little contamination despite stagnation or exposure to variable ambient conditions. In certain cases, it is possible to encounter slightly more moist deposits than normal and Salmonella here.

### 3.2.16. Loading chutes

Deposits are minor here and of the four samples taken, a single one is contaminated with Salmonella.

### 3.2.17. Truck compartments

Generally speaking, the deposits present in these compartments are minor. Where they are more significant, high levels of bacterial flora are observed. This correlates to high moisture levels. Salmonella is detected in three of the ten samples taken.

## 3.3. Level 3 risk

This risk of contamination is attributed to various carriers present in the equipment's environment inside the plant. The key carriers are pests (rodents, insects, birds), occasionally personnel, air and deposits of products. In order to assess the level of contamination affecting the equipment's environment, without taking account of the possibility of transferring contaminants to the product, samples of deposits are taken at various points of the plant outside production lines.

### 3.3.1. Reception pit

The obligatory throughput of all contaminants carried by raw materials, the possibility of numerous deposits, potential access by pests and exposure to atmospheric conditions, make reception pits the perfect place for significant microflora. The results of samples taken from the screens and walls support this hypothesis. In addition to the presence of significant Enterobacteria and Salmonella populations, certainly caused, to a large extent, by raw materials, the presence of a significant quantity of *Clostridium perfringens* should be noted on a site.

### 3.3.2. Inside the plant

Overall, the insides of plants appear not to be greatly contaminated where there are limited quantities of deposits. However, where the deposits are significant, the sample taken is heavily contaminated. In addition, these deposits provide easy food for pests.

### 3.3.3. Bulk loading bay for finished products

The bulk loading bays are open to the outside. When loading trucks, product spillages may occur, resulting in the formation of deposits. A lack of cleaning translates into the presence of a significant number of deposits with a high level of contamination.

### 3.3.4. Ambient air in the plant

The level of air contamination in the plants is assessed during the course of 5 measurement campaigns.

Samples taken outside the plant serve as a benchmark for assessing the extent of contamination inside the plant.

During the five campaigns, the inside of plants is always more heavily contaminated than the outside. In the first two cases, this difference is extremely significant: the outside is 10 times less contaminated than the two points subject to few draughts (press and coater), and 30 to 50 times less than the air intake locations (grinder and cooler intakes).

Moreover, these results show that there is a difference in internal/external flora. The inside of the plant is largely contaminated with *Mucorales*, *Penicillium*, *cladosporiums* and *Rhizopus nigricans*, which are rare or not present outside the plant.

There are slightly more *Aspergillus* inside than outside but, in comparison with other moulds, they appear to be less present in the air.

## 4. Conclusion

The samples of products analysed reveal that the products entering plants are likely to cause significant initial contamination with Enterobacteria and potential contamination with Salmonella.

This study reveals that there may be points inside plants that are likely to pose a significant risk of microbiological growth or contamination: **the filters, the cooler, the locations of prolonged deposits in circuits and in the plant.**

This study should lead every manufacturer to ask themselves questions about contamination at particular points in their plants. An examination of all the program's results should provide them with additional information for analysing their situation. Only a specific study of each site would enable microbiological contamination to be controlled in a plant. In effect, it is only this kind of study that allows us to see, on the one hand, if it is necessary to control key points and, on the other hand, to identify other risks that are inherent to the plant.

## 5. Bibliography

- i'Tec\_S1 of Liaison report no. 40 - May 1995.
- i'Tec\_S2 of Liaison report no. 43 - May 1996
- i'Doc\_S7. Studies of microbial contamination in feed mills. - July 1996