Risiken erkennen – Gesundheit schützen

Bone fragments in beet cossettes

Updated Expert Opinion* No. 005/2005 of BfR of 1 December 2004

Beet cossettes are formed as a by-product when sugar is extracted from sugar beet. They are processed into feedstuffs for food-producing animals and thus also fed to ruminants. Microscopic bone fragments and hair were detected in beet cossettes from Germany. It is not clear how they reached the feedstuff and which species of animal they come from. The detection of bone fragments is an indication of animal protein. With regard to bone fragments from ruminants, the risk of transmission of the BSE pathogen to food-producing livestock has to be examined.

When assessing the BSE risk, the origin of the animal constituents is decisive. The bone fragments may be remains of small animal carcasses or prey. Furthermore, there is a possibility that the contamination of the feedstuff samples can be traced back to the use of organic fertilizer (e.g. meat-bone meal). Although the feeding of animal meal and animal protein to food-producing animals has, in principle, been banned since 2001 because of the risk of BSE transmission, animal meal and bone meal may still be used as fertilisers in agriculture after heat treatment at 133 °C/3 Bar pressure for at least 20 minutes. The precondition is that the animal meal may not contain any risk material from ruminants.

Initial studies only identified rats or mice as the source of animal protein in the beet cossettes. No hereditary material from cattle was detected. In order to provide a definitive answer to the question of origin, BfR examined samples of beet cossettes, in which animal constituents had been detected, using highly sensitive molecular-biological methods. The results confirm the initial examinations. In four out of ten samples examined, hereditary material from rats could be detected, human hereditary material in seven samples and pig-specific DNA in two cases. No genetic material from cattle could be detected. Hence, in the opinion of BfR, there is no BSE risk associated with these beet cossettes contaminated with bone fragments. In principle, however, a risk of food-producing ruminants becoming infected with BSE through the consumption of beet cossettes, which have been contaminated via arable soil with animal constituents, cannot be ruled out bearing in mind knowledge about the persistence of prions in the soil. Based on the current level of knowledge, the risk is deemed to be low. However, it cannot be quantified.

On 10 January 2005 an Expert Meeting[1] was held on the subject in BfR. Discussions focussed on the suitable methods for the analysis of feedstuffs and initial results of the studies. The most recent analytical findings on the incidence of animal DNA in beet cossettes indicate that animal DNA can regularly be expected in potatoes used as feedstuffs.

In beet cossettes from Germany bone fragments and hair were detected microscopically. The impurities are to be found in the lower, still qualitatively detectable range. The source has not yet been definitively clarified.

[1] List of participants at the end of the document.

*Updated on 10 January 2005 and on 27 January 2005

BfR had assessed the following aspects of the findings:

- Morphology and appearance of the bone fragments
- Determination of the animal species in the samples
- Possible input paths
- Risk of BSE transmission

Result

The results of the first studies indicated that the animal impurities were the remains of rodents (rats, mice) which had indirectly reached the raw material during harvesting. According to the current level of knowledge there is no risk of BSE from these impurities. Further studies with highly sensitive molecular-biological methods confirmed the results of the first study. The studies did not supply any indications of cattle remains. However, both human and pig-specific hereditary material could be detected in some of the samples using polymerase chain reaction (PCR).

Reasons

Dried cossettes and beet molasses cossettes as feedstuffs

Dried cossettes are a by-product of food production obtained during sugar extraction from sugar beet. They consist of extracted, dried cossettes (maximum content of ash insoluble in hydrochloric ash: 4.5% dry weight).

Beet molasses cossettes are also a by-product of sugar extraction obtained by drying extracted, molasses-enriched pressed sugar beet pulp (maximum content of ash insoluble in hydrochloric acid: 4.5% dry weight). Both by-products are used, to differing degrees, mainly in rations for ruminants and horses.

Microscopic detection of feedstuffs

The official method for the examination of feedstuffs for animal constituents is microscopic detection (Directive 2003/126/EC).

In feedstuffs animal constituents are accurately identified on a scale of less than 0.1% of the sample using morphological characteristics of animals like bone fragments, muscle fibres, feathers or hair. When bone fragments are present, quantification can be done on the basis of an estimate. The estimate can be validated through control mixtures with defined portions of bone fragments.

Using a microscope it is possible to distinguish between terrestrial animals and fish when detecting bone fragments but no more exact characterisation of animal species is possible. Interlaboratory trials have shown that animal meal from terrestrial animals can be unequivocally detected on a scale of 0.1% of the sample in the presence of fish meal.

The detection of bone fragments is an indication of animal protein. The input of morphologically, non-characteristic animal constituents like, for example, intestines, gelatine or slurry cannot be detected microscopically.

Up to now, soil samples have not been routinely examined microscopically for the incidence of bone fragments. Knowledge obtained from the preparation of feedstuff samples using the official method cannot be simply transferred since suitable concentration methods have not yet been established.

Possible contamination path: soil

Inputs of bone fragments reach the soil in a natural way (e.g. perished animals, excrements) and through land use (e.g. small animals killed during harvesting, biowaste, peat, sewage sludge, animal meal fertilisers).

At Göttingen University, fine sand fractions of various soils were examined microscopically for bone fragments. In addition to loess and alluvial soil from southern and eastern Germany, soil samples were also examined from a so-called "exhaustive test field" (E-field). This E-field had not been fertilised with phosphate-containing substances for 130 years.

In two-thirds of the 200 soil samples examined, portions of bone fragments of on average 0.7% were detected in the fine sand fraction. They were determined both in arable soil and in soil from the E-field. It was not possible to determine the age of the bone fragments. Additional mineralogical studies indicated a soil passage of the bone fragments.

Bone fragments in the fine sand fraction can grow into the epidermis of root crops and tuberous fruits (e.g. sugar beet, celery). As a consequence of whirling up of the top soil through wind, rain or corn threshing, the above-ground parts of plants may also become contaminated with bone fragments or other soil particles.

At Hohenheim University, tests were conducted to determine the extent of the input of bone fragments into soil. Based on population studies in field mice and the bone volume derived from that for 1 hectare topsoil, a theoretical adhesion of 0.0002% to beet cossettes was calculated. Another calculation with similar results was undertaken using animal meal as the fertiliser.

The studies from both universities show that bone fragments are ubiquitous in soil whereby the half-life of the bone fragments fluctuates considerably depending on the respective physico-chemical and biological parameters of the soil. It is not possible to undertake a generally valid quantification of the proportion of bone fragments in soil.

Determination of the animal species in the samples

According to results available to BfR from regional test institutions, hereditary material (DNA) was detected in beet cossettes in a **screening for animal species** using polymerase chain reaction (PCR) analysis which indicates the presence of poultry or mammals. In a follow-up, **animal species-specific PCR analysis,** no DNA from cattle, chickens or turkey could be detected although rodent-specific hereditary material was found.

It is not possible to assess whether the negative findings can be attributed to the absence of ruminant-specific DNA sequences because of the missing data on the detection limits of the PCR analysis used. More particularly, the detection of rodent-specific DNA cannot be seen as evidence of the origin of the bone fragments. This can only be unequivocally determined when the bone fragment fraction is analysed separately. Hence, the rodent-specific DNA could also come from contamination with rodent excrements.

In order to definitively clarify the origin of the contamination, BfR undertook animal species identification using highly sensitive molecular-biological methods. Eleven samples were examined: dried beet cossettes (pelletised) and beet cossettes (enriched or not with molasses). 10 x 200 mg test material was taken from each of the homogenised samples for PCR analysis. After DNA extraction the 10 DNA extracts from a test sample were combined in order to obtain sufficient amounts of DNA for analysis.

The following specific PCR analyses were conducted on the samples:

- Plant-specific PCR
- Specific PCR for cattle
- Specific PCR for rats
- Two different PCR systems specific for mammals

In a DNA amplificability control (possibility of replication), DNA could be detected in all samples using a plant-specific PCR system. In this way, false-negative results as a consequence of a disruption in the reaction stock (inhibition) could be ruled out in the following analyses.

Mammal DNA was detected in all samples. Some of the samples reacted weak-positive to rate DNA in the PCR. The species, cattle, was not detected in any of the samples. The sensitivity of all systems used here was ten genome copies. This means that at least ten genome copies must be present in the reaction stock in order to carry out positive detection. In the case of a theoretical genome size of 0.4 pg for the species cattle, this means for instance that when using a total of 10 ng isolated DNA in the PCR there is a detection limit of 0.04% cattle DNA. Despite the negative results, it cannot therefore be completely ruled out that the samples contain cattle DNA below this detection limit.

The study results presented here are purely qualitative. No quantitative statements can be made because of the analysis used. Furthermore, the isolated DNA amounts were too low in order to use quantative methods. All controls used were flawless. This shows that all analyses were conducted correctly.

In order to identify the animal species, sequential analysis of the DNA segments (amplicons) amplified using the mammal-specific system (*cyt*b gene) was undertaken.

The results, which have still to be evaluated, refer to the overall sample and do not permit any direct conclusions about the origin of the bone fragments. In order to clarify this question, the bone fraction would have to be separated and then analysed. Even if the separation were successful, the DNA may either be eliminated or be so badly damaged as a consequence of the processing of the feedstuff samples that analytical detection using PCR is no longer possible.

Risk of BSE transmission

According to the results of the analysis, there is no risk that ruminants could be infected with BSE through being fed beet cossettes contaminated with these animal remains.

Only rats, mice and pigs could be definitely identified as the source of animal constituents in the beet cossettes. No DNA from cattle was detected. The risk that field rodents could be infected with BSE is deemed to be very low given the absence of any indications of BSE transmission through direct contact with cattle or their excrements (faeces, urine, milk). BSE infection of field rodents from fertilizers used is also highly unlikely today because of the BSE measures introduced several years ago.

Expert Meeting "Bone fragments in beet cossettes"

The Federal Institute for Risk Assessment held an Expert Meeting on 10 January 2005 with representatives from the agricultural test and research institutes, universities and federal research bodies on the findings of bone fragments in beet cossettes. The following agenda items were discussed:

- Microscopic detection of feedstuffs
- Polymerase chain reaction (PCR)
- Possible contamination path soil – initial study results

The results of the meeting are summed up below:

1. Microscopic detection is the official method for the detection of animal constituents in feedstuffs. It permits the detection of characteristic morphological animal structures like bone fragments, muscle fibres, hair and feathers. Using polymerase chain reaction (PCR), the samples deemed to be positive after microscopic examination can be specified in terms of animal species.

2. The bone fragments identified microscopically in feedstuff samples are an indication of the incidence of other animal proteins (remains of small animal carcasses or prey, use of organic fertilizers like meat-bone meal). The origin of the bone fragments cannot be determined microscopically.

3. If no bone fragments have been found microscopically in the feedstuff sample, it is still not possible to make any statements about whether animal, morphologically non-characterisable material is present in the sample or not (e.g. organic fertilizers: slurry).

4. Bone fragments can be found in near surface layers of both unspoilt and cultivated soil. Bone fragments, as constituents of the fine sand fraction, may adhere to harvested and washed potatoes. Furthermore, they can grow into the epidermis of some tuberous fruits and root crops (possible adhesions or incorporations both in feedstuffs and in foods like sugar beet, mangold, carrots, celery, savoy cabbage, silage, hay).
   Arable crops may be contaminated with bone fragments from the topsoil through the whirling up of bone particles by raindrops. Furthermore, a contamination of the harvested crops, for instance through dust development during the threshing of corn or preparation of silage and hay is possible.

5. An estimation of the age of the bone fragments cannot be undertaken using microscopic or PCR methods. Hence, even when bone fragments have been detected in the feedstuff sample, no distinction can be made as to whether these fragments entered the system, soil, before or after the entry into force on the ban on feeding and the regulations concerning specific risk material (SRM).

6. Although DNA analysis does permit a fundamental distinction between different species of animals, it is not possible in individual cases to differentiate between naturally available animal DNA (perished animals) and animal DNA introduced by human beings (animal meal, fertilizers) into the feedstuff sample or the soil.

7. The most recent analytical findings on the incidence of animal DNA in beet cossettes indicate that animal DNA can regularly be expected in potatoes used as feedstuffs. No contamination with cattle DNA has been reported up to now.

8. According to the analytical findings available, there is no identifiable risk that food-producing ruminants can become infected with BSE through intake of the examined beet cossettes. In principle, a risk of contamination with animal constituents from arable land, bearing in mind the persistence of prions in the soil, cannot be ruled out but it can be deemed to be non-quantifiable.

Federal Institute for Risk Assessment
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