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## New Findings concerning “Bovine Meat and Milk Factors” (BMMF)

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In February 2019, the German Cancer Research Center (DKFZ) presented findings on novel infectious agents called “Bovine Meat and Milk Factors” (BMMF). They are said to be found in the meat and dairy products of European cattle. The pathogens ingested through the consumption of these foods in early infancy are said to cause inflammation in the intestinal and breast tissue, which in turn promotes the development of cancer in the surrounding tissue. The disease only manifests itself decades after the actual “infection”. Based on the geographical distribution of new cases of large bowel and breast cancer, the DKFZ suspected a link with the consumption of milk and meat products from European cattle and concluded that infants should not be fed cow's milk too early.

As a result, the German Federal Institute for Risk Assessment (BfR) and the Max Rubner Institute (MRI) came to the conclusion in a joint opinion that the assessment of health risks posed by BMMF was not possible due to insufficient data at the time. Since then, various research groups have dealt with BMMF, which is why both the BfR and MRI have reassessed the topic.

Neither the current nor previous findings support the hypothesis that BMMF is a new type of pathogen. They represent variants of DNA sequences that are already known and in some cases have already been published. Thus far, there is no evidence that BMMF actually cause harmful effects in humans and other organisms. In addition, various current studies show that BMMF are not only found in milk and meat products from European *Bos taurus* cattle, but also in numerous other foods of both animal and plant origin.

The current state of knowledge thus contradicts the hypothesis of the DKFZ, according to which the identified BMMF represent “novel pathogens” that only occur in European cattle and the foods derived from them. The geographical distribution of new cases of large bowel and breast cancer can only be interpreted as an indication of a potential indirect link between the consumption of certain foods and the occurrence of some types of cancer in humans, but does not represent a causal link.

Based on the study results and literature currently available, the BfR and MRI continue to recommend including meat and cow's milk as part of baby food due to their specific micronutrients. Avoiding certain foods in the first year of life is also not recommended with regard to allergy prevention.

In summary, and considering all the data currently available, negative health consequences from BMMF or BMMF DNA contained in meat or dairy products, or other foods, are not expected for consumers of any age.

### 1 Subject of the Assessment

In February 2019, the German Cancer Research Center (DKFZ) announced at a press conference on “Novel Infectious Agents as Cancer Risk Factors” that it had identified a novel “class of pathogens”. According to the hypothesis of the DKFZ, the molecules referred to by

the DKFZ as “Bovine Meat and Milk Factors” (BMMF) would trigger an “infection” in early infancy through the consumption of dairy products and/or beef. These molecules, referred to as “pathogens”, are supposed to induce a chronic inflammatory reaction in certain tissues (bowel, breast), which in turn is supposed to promote the development of cancer in the surrounding tissue (especially large bowel, possibly also breast and prostate cancer), whereby the disease would only manifest itself decades after the actual “infection”. For the reasons described, the DKFZ concluded that there is no direct causality between a BMMF “infection” and, for example, bowel cancer, but that BMMF constitute a share of the bowel cancer risk, which, however, cannot be precisely quantified (DKFZ 2019). This hypothesis originated from epidemiological observations, according to which the geographical distribution pattern of the incidence rates of bowel and breast cancer indicates a close link to the consumption of milk and meat products from European cattle (*Bos taurus*). The BMMF hypothesis is primarily based on the assumption that there is an association between the consumption of food derived from European cattle (species *Bos taurus*) and the development of large bowel and breast cancer (DKFZ 2019; zur Hausen et al. 2017).

The German Federal Institute for Risk Assessment (BfR) and the Max Rubner Institute (MRI) evaluated the corresponding press release from the DKFZ in an initial joint opinion from 2019 (<https://www.bfr.bund.de/cm/343/neuartige-erreger-in-rind-und-kuhmilchprodukten-weitere-forschung-notwendig.pdf>). The opinion found that an assessment of health risks posed by so-called BMMF as cancer risk factors was not feasible at the time due to insufficient data and recommended further research into the suspected link between BMMF and the occurrence of cancer in humans. The need for research described included i.a. the occurrence of BMMF in foods of non-bovine origin, the occurrence of BMMF in healthy people compared to cancer patients, the mechanism of inflammation and cancer induction by BMMF, and studies on the infectivity and inactivation of BMMF in foods.

Various research groups have been working in the field of BMMF since 2019. Neither the current nor previous findings support the hypotheses that BMMF represent a new type of pathogen or have the ability to “infect” animal or human cells. Various current studies also show that BMMF are not only found in milk and meat products from European cattle (*Bos taurus*), but also in numerous other foods of non-bovine origin, both animal and vegetable (Pohl et al., 2022). For these reasons, the BfR and the MRI have carried out a reassessment of the topic based on the available findings from studies and the literature.

## 2 Findings

The circular, single-stranded DNAs detected by the DKFZ do not represent a new class of pathogens, but belong to the widespread CRESS (Circular Rep-Encoding Single-Stranded) DNA group, which has been known for some time (Rosario et al. 2012). BMMF were divided into two groups (BMMF1 and BMMF2) based on their sequence similarity to two previously discovered DNA molecules called “Sphinx” (Slow Progressive Hidden INfections of variable <X> latency) (Manuelidis 2011). Individual BMMF from these groups share up to 98% sequence similarity with the corresponding Sphinx (Funk et al. 2014; Whitley et al. 2014). The remaining identified molecules were identified as small, circular, viral genomes (Gemycircular viruses; group 3) or were distantly related to a plasmid of a *Psychrobacter* species (group 4) (zur Hausen et al. 2017). The BMMF groups therefore do not correspond to any new classes of DNA molecules, but rather represent variants of already known DNA sequences, some of which have already been published.

To date, there are no publicly available studies or data that prove that the BMMF referred to as “pathogens” by the DKFZ actually cause harmful effects in organisms of any kind, i.e. that

they are pathogens at all. Furthermore, there is currently no evidence of a causal relationship between the presence of BMMF in an organism, including humans, and the development of a disease in this organism.

Several recently published studies show that the BMMF most intensively studied by the DKFZ staff, are found not only in foods originating from European cattle (species *Bos taurus*), but also in foods from non-*Bos taurus* species, such as buffalo (*Bubalus bubalis*) (König et al. 2021a), sheep and goats (König et al., 2021b). They have also been detected in all other food groups tested to date, including those of plant origin (Pohl et al. 2022).

The current state of knowledge thus contradicts the main assumption of the hypothesis of the DKFZ, according to which the identified BMMF represent “novel pathogens” that only occur in European *Bos taurus* cattle and derived food products.

The epidemiological observations can only be interpreted as indicating a potential, indirect link between the consumption of certain foods and the occurrence of some types of cancer in humans, but do not represent a causal link. This is all the more true since BMMF were detected in all foods examined (of plant and animal origin) and in numerous other matrices.

### 3 Rationale

#### 3.1 Risk assessment

##### 3.1.1 Possible source of hazards (hazard identification)

According to the documents from the DKFZ press conference (2019), BMMF are “single-stranded, circular DNA elements that are very similar to the sequences of specific bacterial plasmids”. All BMMF known to date each have a gene for the replication initiator protein (Rep protein) required for their own replication, independently of other genes present. According to Eilebrecht et al. (2018), most BMMF show a similarity to plasmids of the bacterium *Acinetobacter baumannii*, whereby some of these molecules also show similarities to certain viruses (Gemycircular viruses) having a small, circular, single-stranded genome (DKFZ, 2019). In addition, it is not believed that they occur in nature as naked DNA, but rather associated with proteins, as is typical for viral genomes, although no evidence of such proteins has yet been published. According to the DKFZ (2019), BMMF “represent a new class of pathogens that, in their characteristics, lie between viruses and bacteria”. In 2019, the DKFZ pointed out that the nature of these molecules, which the DKFZ referred to as “pathogens”, was not clearly defined.

The molecules designated by the DKFZ as BMMF were first described by the American scientist Laura Manuelidis in 2011 (Manuelidis 2011). Two circular DNA molecules were obtained from mouse neuroblastoma cells (N2a) producing the scrapie agent 22L, brain tissue from hamsters infected with scrapie strain 263K and brain tissue from mice infected with FU-CJD (Creutzfeldt-Jakob disease). These DNA molecules have been termed Sphinx (**S**low **P**rogressive **H**idden **I**nfections of variable <X> latency) sequences because they have been demonstrated to be associated with occurrences of infection. According to Manuelidis (2011), the sequences, structures and coding capacities of the Sphinx circular DNA elements are of viral origin. She also points out that Sphinx reside inside the host cell and replicate like viruses. The BMMF were divided into two groups (BMMF1 and BMMF2) based on their sequence similarity to the two Sphinx DNA molecules. Individual BMMF from these groups show up to 98% sequence similarity to the corresponding Sphinx. Both Sphinx sequences

(Sphinx 1.76 and Sphinx 2.36) show high sequence similarities to several subsequently isolated BMMF molecules (Funk et al. 2014; Manuelidis 2011; Whitley et al. 2014). Circular DNA molecules with high similarity to Sphinx 2.36 were also isolated from the serum of healthy cattle (Funk et al., 2014). The remaining molecules were identified as small, circular, viral genomes (Gemycircular viruses) or were distantly related to a plasmid of a *Psychrobacter* species (zur Hausen et al. 2017). The BMMF groups therefore do not correspond to any new classes of DNA molecules, but rather represent variants of already known DNA sequences, some of which have already been published.

In further investigations by the Manuelidis research group (Manuelidis 2019; Yeh et al. 2017), the Rep protein, which is encoded in the Rep gene of the Sphinx 1.76 DNA and shows about 86% amino acid sequence similarity to the BMMF Rep protein investigated by the DKFZ, was identified in various tissues from mice, rats, hamsters and humans. Clear expression of the protein were detected i.a. in colon, arterial smooth muscle and macrophages of healthy mice and in human oocytes and sperm progenitor cells. According to the DKFZ (2019), the BMMF Rep protein has so far been detected in human colon, breast, prostate and brain tissue, an observation consistent with the findings of Manuelidis.

Since all currently known BMMF possess the Rep protein necessary for their own replication, they belong to the so-called “CRESS” class (**C**ircular **R**ep-**E**ncoding **S**ingle **S**tranded) of DNA, which has been known for some time and is widespread in the environment (Rosario et al. 2012; Zhao et al. 2019). In humans, CRESS DNA has been isolated from individual stool samples (Altan et al. 2018; Ng et al. 2015; Siqueira et al. 2018) and from nasal swabs (Altan et al, 2019). They have also been detected in samples from sewage treatment plants (Castrotrignano et al. 2017; Kraberger et al. 2015) and treated wastewater (Rosario et al. 2009a; Rosario et al. 2009b).

Many of the CRESS DNAs identified to date are known to be, or appear similar to, small, circular, viral genomes. Such genomes do not usually occur in nature as naked DNA, but are tightly bound to protective protein molecules. The resulting nucleoprotein particles are very robust to environmental conditions. This could explain the identification of single-stranded DNA (ssDNA) viruses in purified water, which according to Rosario et al. (2009b) suggests that they might be resistant to chlorination. In light of this fact, the significance of the isolation of BMMF DNA from large bowel tissue as reported by the DKFZ is unclear, since it has not been demonstrated that the isolated DNA was originally contained within large bowel cells. The stability of BMMF nucleoprotein particles may allow them to survive passage through the gastrointestinal tract when ingested, although no such published data exist. In this case, their occurrence on the surface of the large bowel epithelium would be expected.

In a joint study by the MRI, the Christian-Albrechts-University zu Kiel and the Ludwig-Maximilians-University Munich, BMMF DNA was detected in 93 of 143 samples examined. The sample material included that taken directly from animals (pig faeces, pig saliva, chicken faeces), as well as samples of animal origin and non-bovine origin such as milk, cheese, sausage and meat from sheep, pig, deer, wild boar, chicken, turkey and duck, as well as fish and fishery products from pangasius, salmon, pollock, walleye, crab, halibut, mackerel and sea bream procured from grocery stores. Furthermore, fruit (apple, pineapple, blackberry, strawberry, tangerine, peach, blueberry, currants), vegetables (lettuce, carrots, peppers, cucumber, fennel, radishes, broccoli, kohlrabi, chickpeas, ginger, tomatoes, onions), nuts and seeds (coconut, walnut, peanut, hazelnut, pumpkin and sunflower seeds) and various types of flour (wheat, rye, corn, oat, lentil, soy, spelt flour) were also investigated. Although only small sample numbers were tested in each case, BMMF DNA was detected in each of the above mentioned food groups (Pohl et al. 2022). The BMMF elements were detected using a

specific polymerase chain reaction (PCR) and DNA sequencing of individual PCR products to confirm detection. It should be noted that no whole genomes were described in this publication.

Two studies by the Ludwig-Maximilians-Universität in Munich (König et al. 2021a; König et al. 2021b) also detected BMMF DNA in milk from water buffalo, as well as in milk from sheep and goats. In the first study (König et al. 2021a), a total of 30 milk samples from two different water buffalo farms in Niedersachsen and Baden-Württemberg were examined using PCR with primers specific for Sphinx and *Genomoviridae*. Positive signals were detected in 26 of these samples; a total of 21 complete circular genomes were obtained. Two circular DNA genomes were detected in each of five samples, although specific DNA sequences were searched for and not single-stranded, circular DNA. This probably underestimated the number of circular DNA elements, and it may have been possible to detect further circular DNA elements had a non-specific approach been used, e.g. with physical / chemical methods for the purification of small particles and non-specific cloning / sequencing. It should be kept in mind that the water buffalo located in Germany could have ingested the DNA elements found in them in a currently unknown way. Studies on the occurrence of the DNA elements in water buffalo located in Asia are not yet available. In a second study by the authors (König et al. 2021b), 73 individual samples of sheep's milk from five different herds, as well as six commercial milk samples, were examined for the presence of Sphinx/BMMF DNA using specific primers, also by means of PCR. In the individual milk samples, Genomoviridae were detected in eight samples from two farms while DNA similar to Sphinx/BMMF was detected in one sample. Sphinx/BMMF DNA was detected in two of the commercial milk samples. Furthermore, 40 individual milk samples from goats from three farms were examined in this study together with six commercial goat milk samples. While no specific DNA elements were detected among the individual milk samples from the goats, *Genomoviridae* were detected in one and Sphinx/BMMF DNA was detected in three other commercial milk samples.

In all studies published to date, a Rolling Circle Amplification (RCA, form of DNA replication in ring-shaped DNA molecules) and then a specific PCR were performed prior to detection of the DNA elements. Two propagation steps are therefore necessary before these molecules can be detected. Although quantitative statements have not yet been published, the data available to date show that the concentration of the molecules in the samples examined is very low.

Although Sphinx/BMMF DNA has been detected in all food groups examined so far, it is hardly possible to directly compare the individual results. The research group led by Manueldis and, to some extent, the zur Hausen research group as well, first isolated virus particles before they specifically amplified circular DNA. Some studies performed random amplification of total circular DNA, others performed Sphinx/BMMF-specific amplification by targeting known DNA sequences. In all cases, multiple amplification steps were performed using RCA and PCR. As there is no standard procedure for conducting the studies, almost all of them vary in the nature of the starting material and the number of individual amplification steps, such that the starting concentrations of the Sphinx/BMMF molecules are unknown. Additionally, none of the published studies make any reference to the potential mutation rates associated with the amplification and sequencing steps. These can, however, have a significant impact on the sequence similarity between the previously isolated Sphinx/BMMF molecules, and for this reason it is difficult to compare the results. Serious differences and uncertainties exist in the detection methodologies. The overall uncertainty concerning hazard identification here is rated as high.

In summary, it can be stated that Sphinx/BMMF DNA is found in numerous human and animal tissues, in the environment, in foodstuffs and in all food groups investigated to date, both of plant and animal origin. Statements on the quantitative distribution (prevalence and concentration) are currently not available for the environment, animal populations, food or animal feed. Furthermore, no data or information is available on the influence of food technology processes on BMMF DNA. Presence in processed (e.g. pasteurized) food has been documented and suggests that BMMF DNA is not eliminated by this type of processing. On the other hand, however, recontamination after heating cannot be ruled out. Studies investigating the impact of different processing technologies on BMMF DNA, e.g. in the form of challenge studies, are currently not available.

### 3.1.2 Hazard potential (hazard characterisation)

Manuelidis (2011) derived the name of the molecules she termed Sphinx from their concentrated occurrence in infectious TSE (Transmissible Spongiform Encephalopathies) preparations. The author concludes that the Sphinx sequences may play a role in TSE infections, although they appear to persist symbiotically and at low levels in normal cells and tissues. She believes that this and other cryptic, circular DNAs could lead to or contribute to neurodegeneration and infection-related tumour transformations. The author does not provide evidence of a causal link between Sphinx and the occurrence of diseases, but points out the need for clarification of any link between the occurrence of infections and the potential role of Sphinx in infection-related tumour formation. Circular DNA molecules, which the authors say are closely related to Sphinx 1.76 (79-98% nucleotide similarity), were isolated from brain tissue from multiple sclerosis patients (in two out of four samples) (Whitley et al. 2014).

Isolation of CRESS DNA has been reported in association with various human diseases such as encephalitis (Phan et al. 2015), periodontitis and respiratory diseases (Abbas et al. 2019), and pericarditis (Halary et al. 2016). Possible involvement in the pathogenesis of the diseases mentioned above was discussed, but not considered proven, since other causes for the occurrence of CRESS-DNA are possible.

Based on epidemiological observations, a link between bowel, breast, prostate and lung cancer with BMMF has been discussed for several years (zur Hausen 2012; zur Hausen et al. 2017; zur Hausen and de Villiers 2015). BMMF are supposed to induce chronic inflammation in precancerous tissues, leading to increased generation of free radicals (e.g. reactive oxygen and reactive nitrogen species) and DNA mutations in replicating cells (e.g. cancer progenitor cells) (zur Hausen et al. 2019). The DKFZ (2019) supports this hypothesis, according to which BMMF molecules induce a chronic inflammatory reaction after their appearance in certain tissues (bowel, breast), which may induce cancer development in the surrounding tissue (particularly for large bowel, and maybe also for breast and prostate cancer). Sections of large bowel tumour tissue with detectable BMMF DNA show elevated levels of reactive oxygen species, which are a hallmark of inflammation. These oxygen radicals favour the development of genetic changes (DKFZ 2019). The consumption of dairy products and/or beef is said to lead to an "infection" with BMMF, especially in early infancy as the immune system is not yet fully developed (zur Hausen et al. 2017). This "infection" in infancy is supposed to result in long-term persistence of infected foci in certain tissue layers of the large bowel. The induction of chronic inflammation with the formation of oxygen and nitrogen radicals is described by zur Hausen et al. (2019) as a specific trigger for random mutation events over a period of about 40 to 70 years. BMMF, therefore, supposedly have an indirect carcinogenic effect. This does not mean that they interfere directly with cancer-promoting molecular processes in the cell, instead they create a largely inflammatory, cancer-promoting environment. For the reasons described, the DKFZ concluded that there is no direct causality between

BMMF and, for example, bowel cancer, but that BMMF constitute a share of the bowel cancer risk, which, however, cannot be precisely quantified (DKFZ 2019).

In 2021, the DKFZ published a new study (Bund et al. 2021) and an associated press release (DKFZ 2021), which claimed that BMMF were detected close to bowel cancer tumours. Bund et al. (2021) detected the BMMF-Rep protein specifically in close proximity to CD68 (macrosialin)-positive macrophages in the interstitial lamina propria bordering large bowel cancer tissue, which the authors suggest indicates the presence of local chronic inflammation. BMMF1 DNA was isolated from the same tissue areas. The researchers showed that both the glycoprotein macrosialin and the BMMF1-Rep protein were present in higher amounts in older bowel cancer patients (n=7) than in younger healthy subjects (n=8). A comparison with subjects of the same age was not carried out. The authors believe the studies support the hypothesis that consumption of milk and beef is causally related to the development of bowel cancer.

Replication of various BMMF in human cells has been demonstrated (Eilebrecht et al. 2018), although this requires cellular proteins that have not yet been identified. Introduction of BMMF DNA into human cells affected the expression of several human genes involved in inflammation (Eilebrecht et al. 2018). Nevertheless, so-called BMMF do not currently appear to have any characteristics specific to them. The only properties that could be demonstrated are also characteristic of the numerous small, circular CRESS DNA molecules that are frequently isolated from organic sources and found in organisms from all three domains of life (bacteria, archaea and eukaryotes) (Krupovic and Forterre 2015). Rep protein genes are found not only in CRESS DNA, but also in many small, circular virus genomes, several of which have already been isolated from human samples (Abbas et al. 2019; Halary et al. 2016; Phan et al. 2015; Uch et al. 2015; Wang et al. 2019; Zhou et al. 2015).

Sphinx 2.36 itself (the classification type for an entire group of BMMF) shares a 67% sequence identity with the genome of a bacterial virus (a bacteriophage) that infects *Acinetobacter baumannii* (Longkumer et al. 2013). Small, circular Rep-encoding DNA molecules have such a long evolutionary history that viral Rep genes have been integrated into the genomes of multiple species of prokaryotes and even into the mitochondrial and chloroplast genomes of some eukaryotes, though not yet in humans (Zhao et al. 2021).

The DKFZ reported that a total of 350 individuals, both healthy and with cancer, carried serum antibodies against a BMMF Rep protein and took this as evidence that these individuals were either currently or previously exposed to BMMF DNA (DKFZ 2019). Due to the large number of small viral DNA molecules, that occur frequently in humans and rely on Rep proteins for their replication, and the fact that there is high homology between many Rep proteins, including with BMMF Rep proteins (Kazlauskas et al. 2018; Villiers et al. 2019), it should be expected that many people will possess antibodies against one or more Rep proteins. Since it is not uncommon for antibodies against a specific antigen to also react with proteins whose amino acid sequence demonstrates a high degree of similarity to the antigen (cross-reactivity), it cannot be determined with certainty whether all of the antibodies detected in the DKFZ studies formed as a reaction to the BMMF Rep protein and not to one of the many possible homologous Rep proteins.

As further evidence of an association between BMMF and bowel cancer, the DKFZ group reported that they detected the BMMF-Rep protein in peritumorous bowel tissue from cancer patients (Bund et al. 2021). Detection of the same protein in germ cells of healthy people and in the brain tumour tissue of a glioblastoma patient was shown by another group two years earlier in the USA (Manuelidis, 2019). Given that BMMF DNA has been detected in all food

groups studied to date and appears to be in a stable complex with specific binding proteins, isolation of BMMF DNA from bowel tissue is not extraordinary and does not automatically indicate a causal relationship between the presence of BMMF and resulting disease.

In conclusion, there is no evidence at this time to support a causal relationship between the presence of BMMF or BMMF DNA in any organism, including humans, and the development of disease in that organism. The data to date should still be interpreted as a provisional indication of a, possibly indirect, link. A causal relationship between the intake of BMMF or BMMF DNA and the occurrence of health effects has not yet been proven. Overall, the available data should be assessed as incomplete and also subject to substantial uncertainties.

### 3.1.3 Exposure assessment

According to zur Hausen et al. (2019), the highest risk of what they call “BMMF infection” is posed by the uptake of molecules during the weaning phase from breastfeeding or when breastfeeding does not occur, since breast milk contains ingredients with e.g. anti-pathogenic properties, which generally prevent infections (Peterson et al. 2013).

In general, the first exposures to BMMF and BMMF DNA could already occur in the first six months of life. Even though the recommendations currently available in Germany stipulate that infants should be breastfed exclusively for the first four to six months of life (Koletzko et al. 2016), representative data on breastfeeding patterns in Germany show that only 68% of women follow this recommendation. 40% of the infants were exclusively breastfed up to the end of the fourth month and 13% up to the end of the sixth month (Brettschneider et al. 2018).

Supplementary food should be started at the earliest from the fifth month of life and at the latest from the seventh month (Koletzko et al. 2016). Data from the **D**O**R**t**M**und **N**utritional and **A**nthropometric **L**ongitudinally **D**esigned (DONALD) study from 2004 - 2012 show that, contrary to this recommendation, about a third of infants were already receiving supplementary foods before they reached the age of four months (Foterek et al. 2014). Due to the composition of the non-representative DONALD cohort, it can be assumed that a higher proportion of families in the overall population do not implement this recommendation and that infants may already be ingesting BMMF or BMMF DNA via supplementary foods before the age of four months. Boiled vegetables are recommended as the first supplementary food for infants, which are gradually augmented with meat and potatoes (vegetable-potato-meat mash). Meat is a relevant supplementary food component necessary for meeting the infant's iron requirements (Kersting et al. 2021; Koletzko et al. 2016). Cow's milk, which represents another possible source of BMMF or BMMF DNA, is also recommended for use in supplementary foods (milk-grain porridge) due to the calcium it contains (Kersting et al. 2021; Koletzko et al. 2016). Avoiding certain foods in the first year of life is also not recommended due to the need for allergy prevention (Kopp et al. 2022). Even if breastfeeding is to continue during and after the introduction of supplementary food (Koletzko et al. 2016), only around 20% of children in Germany are partially breastfed up to the age of twelve months with around 16% fed beyond the age of twelve months (Brettschneider et al. 2018).

Although BMMF and BMMF DNA appear to be widespread in the environment and present in all commonly consumed food products studied (König et al. 2021a; König et al. 2021b; Pohl et al. 2022), there are no studies that provide data on the prevalence or concentrations of these molecules in the various food sources. It is therefore currently not possible to make statements about the intake quantity or frequency of BMMF or BMMF DNA. However, due to the fact that all of the investigated foods – of both animal and plant origin – are affected, it is obvious that all consumers take up these molecules with their food, even if no quantitative



statements can currently be made about the amount of intake. Since BMMF or BMMF DNA have been detected in all foods investigated to date, no particular risk of exposure can be identified from the consumption of certain foods, such as beef or dairy products.

In summary, it can be said that BMMF or BMMF DNA can be found in all foods. However, since no information is available on the prevalence and concentration of these molecules in the various matrices, no precise estimate of human exposure can be made at this time. Therefore, an assessment and evaluation of exposure cannot be undertaken at present due to insufficient data.

#### 3.1.4 Risk characterisation

Risk characterisation of BMMF or BMMF-like DNA in food as possible cancer risk factors cannot be undertaken at the present time due to insufficient data.

Due to the lack of information and valid data, in particular on the prevalence and concentration of BMMF or BMMF DNA in food, it is not possible to estimate consumer exposure to BMMF or BMMF DNA through food consumption, with due consideration to the current state of knowledge. As a result, an exposure assessment is currently not possible. Even though new insights into the occurrence of these molecules in numerous plant and animal foods have been gained in recent years, there are still significant gaps in quantitative data on possible sources of exposure. This information is crucial for understanding the risk of exposure and deriving any necessary risk minimization measures.

Information on the probability of occurrence as well as on the type, duration, reversibility and severity of negative health consequences cannot be given at present either, as no negative health consequences have been demonstrated to date.

Taken together, all currently available data suggests that the molecules known as BMMF and previously known as Sphinx are widespread both in the environment and in all food groups studied to date. At this time there is no data to support a carcinogenic or other adverse effect related to the presence of these molecules in animal or human cells. Scientific findings that suggest such an effect are not to be regarded as sufficiently evident.

In particular, the above-mentioned epidemiological observations can only be interpreted as preliminary indications of a possibly indirect link between the consumption of different foods and the occurrence of some types of cancer in humans, but they have not yet proven a causal link. It cannot be ruled out that an as yet unknown pathogen is the cause of diseases such as cancer or is involved in their development in some way. However, the various studies in recent years on the occurrence of BMMF in different matrices contradict the main assumption of the DKFZ hypothesis, according to which BMMF is a “pathogen” that only occurs in *Bos taurus* cattle or in foods of bovine origin.

There remains a lack of valid, evidence-based studies for assessing any health risk, for example on the prevalence and concentration of BMMF in food. Furthermore, no data or information is yet available on the influence of food technology processes on BMMF or BMMF DNA. The information on the mechanism of possible inflammation and cancer induction by BMMF has so far been of a hypothetical nature. Assessment of a link between the consumption of foods containing BMMF and the occurrence of tumour diseases does not seem possible at the moment because the BMMF mentioned are more or less ubiquitous and are only said to act as indirect carcinogens and after a very long latency period.

In addition, the epidemiological work published to date must be viewed in a cautiously informed manner. In the case of colorectal cancer, previous studies indicate that consumption of red and processed meat correlates with the occurrence of bowel cancer (Chan et al. 2011; Corpet 2011; Huxley et al. 2009; Veettil et al. 2021; WCRF 2018), but that high consumption of milk and milk products is associated with a reduced risk of bowel cancer (Veettil et al. 2021; WCRF 2018). According to the World Cancer Research Fund International (WCRF 2018), neither red meat nor cow's milk consumption leads to an increased incidence of breast cancer. In addition, the evaluation of 21 cohort studies with a total of 1.1 million women shows no link between milk consumption and a risk of breast cancer (Wu et al. 2021). Furthermore, there is currently no evidence for a link between early feeding with breast milk and the cancer risk of the offspring in adulthood. The association with breast cancer has been best studied; however, the results are inconsistent (Diaz-Santana et al. 2020; Ekblom et al. 1993; Freudenheim et al. 1994; Titus-Ernstoff et al. 1998; Weiss et al. 1997; Wise et al. 2009). Some studies indicate a protective effect of breastfeeding (Diaz-Santana et al. 2020; Freudenheim et al. 1994; Weiss et al. 1997).

In summary, and considering all the data currently available, negative health consequences for consumers of any age arising from BMMF or BMMF DNA contained in meat or dairy products, or other foods, are not expected.

### 3.1.5 Assessment of the data quality

Regarding the currently available data, the greatest uncertainties in risk assessment are, on the one hand, the fundamental question of evidence for a causal link between the intake of BMMF or BMMF DNA via food and the occurrence of diseases in humans, and, on the other hand, the exposure estimate. These two aspects also have the greatest impact on the assessment of any health risk. Uncertainties connected with the reported detection methods should also be rated as high.

## 3.2 Framework for action, recommendations for measures

Based on the current evidence, the presently valid dietary recommendations do not need to be modified.

The recommendations currently available for breastfeeding and the introduction of supplementary foods, which stipulate that infants should be exclusively breastfed for the first four to six months of life, continue to apply. Breastfeeding should also continue during the introduction of solid food, at the earliest from the fifth month of life and at the latest from the seventh month. The total duration of breastfeeding is decided by mother and child. Meat and cow's milk are explicitly recommended as supplementary foods due to their specific micronutrients (especially iron and calcium). A weekly intake of approximately 150 g of meat and a maximum daily intake of 200 ml of cow's milk are considered adequate (Kersting et al. 2021; Koletzko et al. 2016). Avoiding these foods is not recommended in the first year of life. Avoiding certain foods in the first year of life is also not recommended due to the need for allergy prevention (Kopp et al. 2022).

For adults, the German Society for Nutrition recommends meat consumption of 0 to 600 g per week and daily consumption of milk and milk products (Deutsche Gesellschaft für Ernährung (DGE) 2017).

## 3.3 Other aspects

A health assessment of BMMF or BMMF DNA is currently not possible due to insufficient data and the associated uncertainties. The gaps identified in the data should be bridged by appropriate investigations. Regarding food hygiene, this applies in particular to representative data on the prevalence and concentration of BMMF and BMMF DNA in various food groups. With regard to the epidemiological information mentioned in the text, further studies of the occurrence of BMMF in foods of animal origin derived from species (e.g. *Bos indicus*) other than *Bos taurus*, would be particularly useful. Furthermore, studies on the influence of food technology processes on these molecules would be of interest. The detection methodology should be further developed and standardised, or detailed specifications should be drawn up for the documentation of the methods used in studies, making evaluation or a comparative consideration of the literature possible. The information on the mechanism of possible inflammation and cancer induction by BMMF should be examined in further studies that take into account causality criteria.

**Further information on the subject of BMMF from the BfR website:**

Opinion No. 14/2019 “New pathogens in beef and cow's milk products: More research required“: <https://mobil.bfr.bund.de/cm/349/new-pathogens-in-beef-and-cows-milk-products-more-research-required.pdf>

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The German Federal Institute for Risk Assessment (BfR) is a scientifically independent institution within the portfolio of the German Federal Ministry of Food and Agriculture (BMEL). The BfR advises the Federal Government and the States ('Laender') on questions of food, chemicals and product safety. The BfR conducts independent research on topics that are closely linked to its assessment tasks.

## About the MRI

The Max Rubner Institute (MRI) focusses its research on consumer health protection within the food sector. As a federal research institute within the portfolio of the German Federal Ministry of Food and Agriculture (BMEL), the Max Rubner Institute advises the federal government on a scientific basis on all questions relating to nutrition and food. The MRI has offices in Kiel, Kulmbach and Detmold, and its headquarters is located in Karlsruhe.

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