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Micro-ribonucleic acid in milk: health risk very unlikely

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Ribonucleic acid (RNA) occurs in animal and plant cells and has many biological functions. RNA plays a central role in the reading of genetic material, thereby ensuring that important substances are produced for the cells. Among other things, it also ensures the formation of the proteins needed by the cells. There are different types of RNA with different functions.

One type is micro-RNA (miRNA), and its job is to regulate numerous processes in a cell. It has been suggested, however, that some of these miRNAs are involved in the emergence of tumours and other health problems.

The German Federal Institute for Risk Assessment (BfR) was requested to assess the potential health risks of the miRNAs contained in cows' milk and dairy products. Data on such factors as the intake of miRNAs are urgently needed for a definitive risk assessment, but no such data are available at this point in time. The data that are currently available do not permit the conclusion that miRNAs in milk pose a health risk.

Based on the available data on miRNAs, the BfR views it as highly unlikely that the miRNAs ingested with milk have any effect on human health. Current scientific knowledge does not supply any grounds to advise the general population to refrain from consuming milk and dairy products in the recommended quantities and amounts that are common in Germany.

Opinion on the possible health risks of the micro-RNAs contained in milk and dairy products

1 Subject of the opinion

The Federal Ministry of Food and Agriculture (BMEL) in Germany officially requested the German Federal Institute for Risk Assessment (BfR) to compile an opinion on the possible health risks of micro-RNAs (miRNAs) contained in milk and dairy products. One researcher believes that there is a connection between the intake of miRNAs via cows' milk and various disease patterns. He proposes a number of measures, including a ban on pasteurised milk. The view of this researcher is addressed in the following.

2 Findings

The assessment of health risks generally comprises (1) the identification and characterisation of the hazard potential as a qualitative and / or quantitative assessment of the harmful impact on health that may result from the source of the risk as well as (2) the assessment of exposure levels and, finally, (3) the characterisation of the concrete risk ("*risk characterisation"*).

As no robust data are available either for the identification and characterisation of the hazard potential or for an assessment of exposure levels, and given the major uncertainties associated with any assessment, it is currently not possible to qualitatively or quantitatively assess the frequency and severity of any harmful effects on health resulting from the intake of miRNAs via cows' milk.

The postulated hypotheses on the potential health risk due to the consumption of cows' milk are primarily based on the interpretation of study results from basic research that are the subject of controversial debate in the scientific community as well as on selectively chosen investigations. This also applies to the use of epidemiological studies, which are not suited to the purpose of showing causal relationships.

Based on the data that are currently available on the stability of miRNAs and their oral bioavailability as well as on possible effects and the cellular concentrations of miRNAs that would be necessary for these effects, it is to be assumed that it is very unlikely that the miRNA consumed with milk has any effects on human health. Current scientific knowledge does not supply any grounds to advise the general population to refrain from consuming milk and dairy products in the recommended quantities and amounts that are common in Germany.

3 Reasons

3.1 Risk assessment

3.1.1 Potential source of risk (agent)

MiRNAs are ubiquitously found in the tissues of plants and animals. They play a central role in the regulation of gene expression in the cell. MiRNAs regulate numerous physiological processes, but it has been suggested that some miRNAs are associated with the emergence of tumours and other disease patterns. It is basically possible for miRNAs from a specific species to have an effect in cells of a different species to the extent that corresponding sequences are present in the genetic material of the target organism.

Humans ingest countless different miRNAs every day via the food they consume. These miRNAs may come from plant or animal sources. Depending on the manner in which food is prepared, it must be assumed that a part of the miRNAs contained in the food is already destroyed during the preparation process. This kind of partial destruction of contained miRNAs during pasteurisation has, for example, already been demonstrated based on two selected miRNAs (Howard et al. 2015).

There are currently no validated analytical methods for the quantification of miRNAs in food matrices including milk and dairy products. To the extent that such data are available, therefore, the validity of published data on the levels of individual miRNAs or total miRNA in milk should be viewed with caution. In this context, it is worthy of note that analysis of the total miRNA concentration in cows' milk by scientists from the same working group resulted in clearly divergent findings in two different publications: Analyses performed by Baier *et al*. (Baier et al. 2014) showed a concentration of specific miRNA-29b of 680 picomoles per litre, whereas a publication by Howard *et al*. (Howard et al. 2015) listed the concentrations of the same miRNA as less than 50 femtomoles per litre. This is equivalent to a difference of more than four orders of magnitude, and it is unlikely that this difference is due solely to the biological fluctuations of substance concentrations in a natural foodstuff like milk – and that it is probably at least partly due to limitations in the analytical methods used.

Notwithstanding the uncertainties with regard to the quantification of miRNAs in foods and the uncertainty over the extent of miRNA losses during the processing of foods, it is to be assumed that functioning miRNAs are in principle ingested with food.

3.1.2 Hazard potential

3.1.2.1 Bioavailability of orally ingested miRNAs

In addition to the losses of miRNAs in food due to various processing stages, it must be assumed that further miRNA molecules are chemically or enzymatically degraded during the passage of food through the gastrointestinal tract of the mammalian organism. No reliable quantitative data are available on this process, however. In principle, it is possible that the "packaging" of miRNAs in milk in small vesicles could result in altered stability in the gastrointestinal tract compared to plant miRNAs, which are not vesicle-packaged in this way. However, the data on the stability and resorption of these vesicles in the gastrointestinal tract is inconsistent. Although data from a study by Title *et al.* (Title et al. 2015) show that certain miRNAs can be detected in the stomach of animals fed with milk, the levels found in the gut content were far lower. Incubation of curdled milk from the stomachs of suckled new-born mice with intestinal fluid showed only low stability of milk miRNAs in the intestinal fluid, which means it must be assumed that the major part of the miRNAs still present in the milk is rapidly degraded in the intestine and only relatively low amounts of functional miRNAs can come into contact with the cells of the intestinal epithelium.

The argument in support of the bioavailability of miRNAs from milk and the resulting effects is based on publications by the group headed by Janos Zempleni (Baier et al. 2014; Manca et al. 2018; Wang et al. 2018). The available literature on this topic is cited by the group in a highly selective manner. Moreover, the only publications mentioned are those that support this line of argument. This method of using published scientific data is not suitable as a basis for arriving at robust conclusions. Other studies that are not mentioned clearly reject the idea that orally ingested miRNAs from milk are bioavailable. In this context, it is pertinent to highlight a publication by Auerbach *et al.* (Auerbach et al. 2016): Among other things, the authors of this study re-analysed the original samples from a study by the Zempleni group (Baier et al. 2014) and were unable to find any indication that miRNAs from milk were bioavailable in humans (Auerbach et al. 2016). The authors of the latter study discuss various reasons for the findings of Baier *et al.* (Baier et al. 2014), including inadequate normalisation processes during data analysis. As the original publication of Baier *et al.* (Baier et al. 2014) contains no information whatsoever on the variance of the data, it is also unclear whether or not the increase in certain miRNA blood levels in humans due to the consumption of cows' milk as reported in the study meet the criterion of statistical significance.

In an animal experiment, new-born mice of genetically modified strains that cannot form certain miRNAs themselves were suckled by wild-type mice who can form normal levels of these miRNAs (Title et al. 2015). Despite the intake of miRNAs via the milk from the mother mice, the study was unable to find any indication of a transfer of the miRNAs from the milk to the blood or tissues of the new-born animals (Title et al. 2015). A further study was performed with genetically modified mice who form an increased amount of a specific miRNA and who therefore produce milk containing far higher levels of this miRNA (Laubier et al. 2015). The results of this study show that, although greatly increased levels of the miRNA in question was found in the milk and in the stomach of suckled young animals, there was no difference in the miRNA levels in different tissues regardless of whether the miRNA-rich milk of genetically modified mother animals was used or the normal milk of genetically non-modified mother animals (Laubier et al. 2015).

The potential bioavailability of plant miRNAs and the possible health-related effects resulting from this bioavailability was the subject of intensive discussion and research some years ago. A 2012 publication by Zhang *et al.* describes the detection of orally ingested plant miRNAs in the blood and tissue of laboratory rodents as well as human blood (Zhang et al. 2012). This publication also reports on the regulation of a gene of the mammalian organism by a plant miRNA administered via the consumed food. Subsequently, however, numerous scientists criticised the results of this study as being non-reproducible and possibly based on contamination artefacts (Dickinson et al. 2013; Tosar et al. 2014). A summary of the scientific discussion on this topic can be found in a review article by Yang *et al.* (Yang et al. 2015). The authors of this publication arrive at the conclusion that it is highly likely that there is no relevant intestinal absorption of orally ingested miRNAs in healthy consumers. The view that the oral bioavailability of orally ingested miRNAs is extremely low is also shared by the BfR Committee for Genetically Modified Food and Feed, which looked at the issue of gastrointestinal RNA absorption in the context of an miRNA-producing genetically modified plant at its meeting on 24 November 2015 (the minutes of the meeting are available in German on the Internet at www.bfr.bund.de/cm/343/9-sitzung-der-bfr-kommission-fuer-genetisch-veraenderte-lebens-und-futtermittel.pdf). An overall review of the published data on the bioavailability of plant miRNAs suggests that it is unlikely that there is any relevant level of absorption in the human body.

It should be noted in this connection that effects of orally ingested plant RNAs have indeed been observed in certain insects (Bolognesi et al. 2012). However, there are major differences between these insects and mammals in terms of the structure of their gastrointestinal tract and the chemical and enzymatic conditions present in the tract as well as with regard to the readiness of intestinal epithelium cells to absorb RNAs – which means that these findings cannot be considered to be directly transferable to humans (on this question, reference is also made to the aforementioned assessment of the BfR Committee for Genetically Modified Food and Feed).

3.1.2.2 Possible effects of orally ingested miRNAs on human health

The preconditions for an effect of externally administered miRNAs in the human organism would be a sufficiently high active level of the miRNAs in question in the blood as well as quantitatively sufficient absorption of the miRNAs from the blood by the cells of any target tissue. No systematic studies have been conducted on the concentrations of a broad spectrum of miRNAs in different tissues that would be necessary for efficient gene regulation. A publication by Title *et al.* (2015) determined the amount of a specific miRNA needed to exercise an effect on the regulation of its target genes in isolated liver cells. In this *in vitro* study, effects were shown from a concentration of this miRNA of roughly 10^4 copies per cell. The authors specify the sensitivity of their detection method for miRNAs in cells as roughly three copies per cell and conclude that, even if a low amount of miRNAs from milk below the analytical detection limit were bioavailable, this small amount of miRNAs would not be expected to produce any physiological effects (Title et al. 2015).

A review article by Yang *et al.* (Yang et al. 2015) also concludes that, to the extent that miRNAs are ingested with food at all, the expected level of exogenous miRNAs in the human body is too low to have any biological effect. At its meeting on 24 November 2015, the BfR Committee for Genetically Modified Food and Feed also arrived at the conclusion that exogenous miRNAs are very unlikely to have any effect on human health (the minutes of the meeting are available in German on the Internet at www.bfr.bund.de/cm/343/9-sitzung-der-bfr-kommission-fuer-genetisch-veraenderte-lebens-und-futtermittel.pdf).

Knowledge based on long history of the use of plant or animal foods does not point to any health risks as a result of the oral ingestion of miRNAs. The cited studies by the group headed by Janos Zempleni (Baier et al. 2014; Manca et al. 2018; Wang et al. 2018) do not describe effects of the consumption of milk and the miRNAs contained therein on human health.

The statements on possible connections between miRNAs from milk and various disease patterns are of a hypothetical nature. They are based on epidemiological studies focusing on observation of humans under real environmental conditions. This means that they are fundamentally different from experimental studies. Epidemiological studies cannot be used to prove that a risk factor is the cause of a disease. Rather, what these studies do is to look for a statistical relationship between exposure to a risk factor and a cause of disease. Moreover, it should be noted that, even if there is a causal relationship between milk consumption and a certain disease pattern, it would still not be clear whether the cause is to be found in miRNAs or possibly in other ingredients of such a complex foodstuff as milk. By the same token, this also means that it is not possible to draw any conclusions regarding the meaningfulness and possible efficacy of any techniques to reduce the level of miRNAs in milk.

3.2 Conclusion

The assessment of health risks comprises (1) the identification and characterisation of the hazard potential as a qualitative and / or quantitative assessment of the harmful impact on health that may result from the source of the risk as well as (2) the assessment of exposure levels and, finally, (3) the characterisation of the concrete risk (“*risk characterisation”*).

Public debate is currently ongoing with regard to a number of possible harmful effects on health due to the supposedly identified “source of hazard of miRNAs in milk”. The objective data available in the published literature on possible connections between miRNAs in milk and various disease patterns are, however, contradictory, and cannot be described as robust. Neither do the cited studies by the group headed by Janos Zempleni (Baier et al. 2014; Manca et al. 2018; Wang et al. 2018) describe any undesirable effects on human health due to the consumption of milk and the miRNAs contained therein. Equally, the knowledge based on the long history of the use of plant or animal foods does not point to any health risks as a result of the oral ingestion of miRNAs. Epidemiological studies are often cited, but such studies are not suited to the purpose of showing causal relationships. Moreover, it is as yet very difficult to assess the influence of other ingredients of the complex food matrix “milk” that may be the causal factor or partly responsible for any effects that are observed. At the current point in time, therefore, the available data are not sufficient to allow qualitative and/or quantitative assessment of a potential harmful effect on health that may result from the hazard source (identification and characterisation of the hazard potential), in particular with regard to quantitative dose-response relationships (minimal dose of miRNAs for the triggering of an effect *in vivo*).

There is also only very limited data for the qualitative and/or quantitative assessment of orally ingested amounts of miRNA from milk for the purpose of exposure estimation. In this connection, uncertainties regarding the quantification of miRNAs in food matrices including milk and dairy products also play a role, as there are currently no validated analytical methods available. The validity of published data on the levels of individual miRNAs or total miRNA in milk – where these data are available at all – should therefore be viewed with caution. Analyses of the total miRNA concentration in cows’ milk by scientists from the same working group resulted in clearly divergent findings in two different publications with a difference of more than four orders of magnitude, for example (Baier et al. 2014; Howard et al. 2015). This discrepancy points to limitations in the analytical methods that are currently available. Although it is to be assumed in principle that functional miRNAs may be ingested with food, the available data are not suitable to allow robust quantitative assessment of the orally ingested amounts of miRNAs from milk for the purpose of exposure assessment. Not only are miRNAs in food lost prior to consumption as a result of various processing steps; the passage of the food through the gastrointestinal tract of a mammalian organism also leads to significant degradation of any miRNAs that have been orally ingested. Reliable quantitative data on these processes are currently not available, however. The possible bioavailability of miRNAs of this type via plant foods has also been the subject of controversial debate. The collection of studies selectively cites several publications, above all by the group headed by Janos Zempleni

(Baier et al. 2014; Manca et al. 2018; Wang et al. 2018). In contrast, other authors come to the conclusion that it is highly likely that no relevant levels of orally ingested miRNAs from plant sources or from milk are absorbed by the intestine of humans (Auerbach et al. 2016; Dickinson et al. 2013; Laubier et al. 2015; Title et al. 2015; Tosar et al. 2014; Yang et al. 2015).

As no robust data are currently available either for the identification and characterisation of the hazard potential or for an assessment of exposure levels, and given the major uncertainties associated with any assessment, it is currently not possible to qualitatively or quantitatively assess the frequency and severity of any harmful effects on human health. Overall, however, the available data indicate that it is very unlikely that the miRNAs consumed with milk have any effects on human health.

More information on this topic on the BfR website …

BfR Opinion on the connection between milk consumption and the development of type 2 diabetes mellitus:

https://www.bfr.bund.de/cm/349/assessment-of-a-possible-connection-between-milk-consumption-and-the-development-of-type-2-diabetes-mellitus.pdf

BfR Opinion on the Transfer of aflatoxins to milk, eggs, meat and offal:

https://www.bfr.bund.de/cm/349/transfer-of-aflatoxins-to-milk-eggs-meat-and-offal.pdf

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About the BfR

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

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