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Vice-President of the EU Commission
Mr Frans Timmermanns

European Commissioner for Health & Food Safety
Ms Stella Kyriakides

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No deregulation of CRISPR/Cas organisms in the EU through the backdoor!

Dear Vice-President Frans Timmermans, Dear Commissioner Stella Kyriakides

We have become aware that DG SANTE is taking a position which is in contradiction to EU GMO regulation, and which may seriously damage consumers, retailers, food producers, farmers and breeders.

In letters to stakeholders and experts from Member States[1], DG SANTE is creating the impression that eggs and hens produced from breeding with transgenic chickens could be marketed in the EU without undergoing the mandatory approval process, risk assessment or labelling.

The specific case concerns (i) transgenic hens used for breeding and (ii) laying hens for egg production and (iii) the eggs produced thereof: the transgenic female chickens for use in breeding are genetically engineered with CRISPR/Cas. The new genomic engineering technique is used to insert a lethal gene into the Z-Chromosome which is transferred to male offspring only. The male embryos are meant to die at an early stage of development. At the same time, the female offspring will supposedly not carry the lethal gene. A patent has already been filed for the technology.[2]

1 https://www.abl-ev.de/fileadmin/Dokumente/AbL_ev/Neu_Themen/Gentechnikfrei/Schreiben_DG_Sante_an_BVL_15.07.2021.pdf
2 https://patentscope2.wipo.int/search/en/detail.jsf?docId=WO2020178822

As shown, DG SANTE assumes that – based on information provided – the female offspring of the transgenic chickens can be used for egg production without undergoing further risk assessment or being labelled. This assumption seems to be based on the claims made by vested interests looking to profit from the patented technology. They state that only the male offspring of the transgenic chicken will carry the lethal transgene, while the laying hens and their eggs will supposedly not carry the transgene. They also claim that the technology is 100 percent safe. These statements appear to be sufficient for DG SANTE to exempt the laying hens and their eggs from mandatory approval process and labelling in the EU.

In private meetings, the position of the EU Commission may be understood by the patent applicants and their associated partners, licensees and clients to mean that they can now start to directly import laying hens and eggs into the EU. The DG SANTE opinion could thus contribute to a situation where the laying hens and eggs enter the EU market unnoticed, without undergoing an approval process. This would profoundly violate EU GMO regulation and pose a serious threat with far-reaching consequences for consumers, retailers, food producers, breeders and farmers. In circumventing and disregarding the legally required standards, including the precautionary principle, DG SANTE is acting beyond its legal competence, and thereby exposing the market to a high degree of legal uncertainty.

The position of DG SANTE not only constitutes a breach of the law, it also stands in contradiction to biological facts:

1. The legal requirements

Articles 3(1) and 15(1) of Regulation (EC) No 1829/2003 apply to genetically modified organisms (GMOs) for food or feed use, to food and feed containing or consisting of GMOs, and to food, food ingredients and feed produced from GMOs. Accordingly, in order to determine whether a product (laying hens and their eggs in this case) is subject to that regulation, it needs to be ascertained whether it consists of GMOs, and, if that is not the case, whether it is produced from GMOs.

In the case of the laying hens, they are the direct female offspring (F1) of the transgenic chickens. They inherit (regardless of whether the transgene works as supposed) genetic material from the mother hens which also will be transferred to the eggs. Thus, there can be no doubt that the laying hens and the eggs produced, are products of GMOs and consist of GMOs. As can be seen with oil, starch or sugar produced from GM plants, it is the production process which is the decisive criterion for the implementation of EU law and not the presence of genetically modified material in the end product.

2. The biological facts

CRISPR/Cas is known to produce both intended and unintended genetic changes. Therefore, not only the intended characteristics, but also the unintended effects have to be taken into account in the risk assessment and approval process. The unintended effects caused by new genomic techniques create new and specific risks. Both intended and unintended genetic changes can result in plants or animals that have genotypes and biological characteristics which are unlikely to arise from chemical and physical mutagenesis. DG SANTE has repeatedly denied these facts (see, for example, DG Sante 2022), its position, however, is not backed by the science:

a) The CRISPR/Cas techniques can override the natural mechanisms in genome organisation that protect essential genes (Belfield et al., 2018; Frigola et al., 2017; Halstead et al., 2020; Kawall, 2019; Monroe et al., 2022). As a result, novel genotypes and biological characteristics can emerge

from applications of this technology. These observations are relevant to both intended and unintended effects.

b) Furthermore, the CRISPR/Cas machinery is known for potentially confusing target regions with specific off-target regions, besides causing unintended insertion of additional genes, decoupling of genes and other specific genomic alterations (of categories such as inversions, deletions or rearrangements) which are unlikely to emerge from spontaneous mutations or physical and chemical mutagenesis (see, for example, Biswas et al., 2020; Braatz et al., 2017; Höijer et al., 2022; Kawall, 2021). In some cases, unusual patterns of inheritance were also observed, thus escaping the Mendelian rules (Höijer et al., 2022).

c) Breeding hens pass on a complex genetic construct to their offspring – this construct is composed of a gene for the CRISPR/Cas machinery and specific genes to activate the gene scissors. The inherited genetic construct may interact with various genetic or epigenetic factors and cause additional uncertainties: for example, to the best of our knowledge, it cannot be ruled out that the gene scissors are activated by factors other than the intended trigger (blue light); if the gene scissors are activated in (some of) the germ cells, this will unintentionally lead to individual offspring with specific, hazardous and unintended genetic changes outside the Z chromosome that could go unnoticed.

The abovementioned biological findings are relevant in this context: laying hens are the direct offspring (F1) of the transgenic hens after crossing with a conventional rooster. Each female offspring may be regarded as a unique individual in regard to its genomic variations. As shown above, each individual F1 chicken may inherit specific unintended effects caused by the process of genetic engineering, which are sited in genomic regions outside the Z chromosome. These effects may also affect the quality and safety of the eggs.

As previously mentioned, the stakeholders looking to profit from the patented technology claim it has a 100 percent success rate. But as we all know, there is never 100 percent precision and predictability in biological processes and living organisms.

It should not be overlooked that what is called ‘segregation breeding’ requires several generations of crossing and selection, together with controls to make sure that no unintended genetic alterations are transferred to the next generations. While the transgenic chickens used for breeding can be subjected to more detailed examination and gene sequencing, it is much more difficult to implement comprehensive control for *all* of the individual female progeny used as laying hens in egg production. In addition, DNA sequencing of the breeding hens may not be sufficient to, for example, exclude unintended biological activation of the gene scissors via epigenetic effects. As a result, the process causes uncertainties which require detailed risk assessment, traceability and effective monitoring.

3. Conclusions and demands

The EU Commission should

- restore legal clarity and certainty in regard to laying hens and eggs produced from transgenic chickens;
- carefully avoid any ‘backdoor’ deregulation of the CRISPR/Cas technology via the introduction of case-specific decisions not sufficiently backed by EU regulations;

- no longer deny that CRISPR/Cas applications (and other new genomic techniques) can cause new and specific risks through intended and unintended effects, and also take the recent scientific findings into account;
- no longer follow an agenda of deregulating GMOs derived from new genomic techniques, and also make sure that the precautionary principle is respected as the underlying principle in its future decision-making.

In addition, we encourage the EU Commission to develop reliable and robust criteria to be applied in regard to benefits such as those in the case of the transgenic chickens. These criteria should also encompass in-depth consideration of the alternatives.

With kind regards

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References:

Belfield, E.J. et al. (2018) DNA mismatch repair preferentially protects genes from mutation. Genome Res, 28: 66–74. http://genome.cshlp.org/lookup/doi/10.1101/gr.219303.116

Biswas, S. et al. (2020) Investigation of CRISPR/Cas9-induced SD1 rice mutants highlights the importance of molecular characterization in plant molecular breeding. J Genet Genomics, 47(5): 273-280. https://doi.org/10.1016/j.jgg.2020.04.004

Braatz, J. et al. (2017) CRISPR-Cas9 targeted mutagenesis leads to simultaneous modification of different homoeologous gene copies in polyploid oilseed rape (*Brassica napus*). Plant Physiol, 174: 935-942. https://doi.org/10.1104/pp.17.00426

DG SANTE (2022) Your letter on CRISPR/Cas techniques create potential new hazards due to unintended effects, reply to Testbiotech. https://www.testbiotech.org/content/communication-testbiotech-eu-commission-risks-new-ge-december-2021-february-2022

Frigola, J. et al. (2017) Reduced mutation rate in exons due to differential mismatch repair. Nat Genet, 49: 1684-1692, https://doi.org/10.1038/ng.3991

Halstead, M.M. et al. (2020) A comparative analysis of chromatin accessibility in cattle, pig, and mouse tissues. BMC Genomics, 21: 698. https://doi.org/10.1186/s12864-020-07078-9

Höijer, I. et al. (2022) CRISPR-Cas9 induces large structural variants at on-target and off-target sites in vivo that segregate across generations. Nat Commun, 13: 627. https://doi.org/10.1038/s41467-022-28244-5

Kawall, K. (2019) New possibilities on the horizon: genome editing makes the whole genome accessible for changes. Front Plant Sci, 10: 525. https://doi.org/10.3389/fpls.2019.00525

Kawall, K. (2021) The generic risks and the potential of SDN-1 applications in crop plants. Plants, 10(11): 2259. https://doi.org/10.3390/plants10112259

Monroe G., et al. (2022) Mutation bias reflects natural selection in *Arabidopsis thaliana*. Nature, 602: 101-105. https://doi.org/10.1038/s41586-021-04269-6

Testbiotech (2021) CRISPR/Cas techniques create potential new hazards due to unintended effects, Letter to the EU Commission, https://www.testbiotech.org/content/communication-testbiotech-eu-commission-risks-new-ge-december-2021-february-2022