Assessment of the technical dossier related to EFSA/GMO/NL/2010/78

Submitted to

Direktoratet for naturforvaltning

by

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Konklusjon i norsk

Vi har etter en nøye gjenomgang av de innsendte "dossiers" som omhandler godkjenning av MON87705, identifisert flere alvorlige svakheter. I søknaden er det en rekke uriktige antagelser, mangel på nødvendig informasjon og svakheter i studiedesign og/eller i valg av metoder som gjør at man ikke har mulighet til å oppdage utilsiktede effekter ved bruk av MON87705. Det foreligger ingen eller mangelfull informasjon om uønskede effekter. I sum støtter ikke dette søkers konklusjon om sikkerhet ved bruk og dyrkning av MON87705.

Dette gjør at produsentens konklusjonen om at sikkerheten ved bruk av MON87705 er ivaretatt, ikke er tilstrekkelig dokumentert.

Produsenten har ikke adressert flere viktige helseaspekter ved introdusering av MON87705 i matkjeden. Det er ikke fremvist tilstrekkelig dokumentasjon på at de nye dsRNA uttrykt i soya MON87705 ikke har andre utilsiktede effekter på andre genutrykk eller at det ikke oppstår andre metabolske forandringer. Det er oppsiktsvekkende og av stor betydning at produsenten ikke har undersøkt og utelukket at det er et lavt-utrykk av små peptider fra det introduserte dsRNA. Søker har bare argumentert for at slike peptider ikke er tilstedet uten at det er fremlagt vitenskapelige bevis for dette. Dette viser at den molekylære beskrivelsen av MON87705 er utilstrekkelig for at man kan utelukke nye proteinbaserte uønskede effekter som kan utøve en risiko for konsumentens helse eller for miljøet.

I henhold til genteknologiloven Vedlegg 4 del V "*Samfunnsmessige fordeler og ulemper* " ligger det til grunn at samfunnsmessig nytte skal tillegges vekt. Det er imidlertid høyst tvilsomt om de endringene i fettsyreprofilene til MON87705 forårsaket av genmodifisering, faktisk er etterspurt eller nødvendige i den norske dietten. Dette må vies ytterlig oppmerksomhet.

Til sist ønsker vi at det vurderes om løsningene på kostholdsrelaterte helseproblemer, spesielt de som er forårsaket av eksponering til transfett, er best løst ved bruk av moderne bioteknologi i nære og fjerne agrosystem, eller om man kan oppnå det samme med et engasjement til sunn mat, kotsholdsopplysning og sosiale programmer som oppmuntrer til et sunt kosthold.

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The following information is respectfully submitted for consideration in the evaluation of product safety and corresponding impact assessment of MON 87705, setting out the risk of adverse effects on health and the environment and other consequences of proposed release under the pertinent Norwegian regulations.

This submission was built in large part using the **Biosafety Assessment Tool** (https://bat.genok.org/bat/) produced by the University of Canterbury and GenØk – Centre for Biosafety. This is a free-to-the-public resource for hazard identification and risk assessment of genetically modified organisms.

All page numbers refer to the document Part 1 of the technical dossier "Application for authorization to place on the market MON 87705 soybean in the European Union, according to Regulation (EC) No 1829/2003 on genetically modified food and feed" submitted by the Developer.

This submission is structured to provide reference to specific provisions for an impact assessment required under the Norwegian Gene Technology Act of April 1993, focusing on the requirements in Appendix 2 - Principles for environmental risk assessment pursuant to sections 13-16 of the regulations and Appendix 4 - Evaluation of ethical considerations, sustainability and benefit to society, cf section 17 of the "Regulations relating to impact assessment pursuant to the Gene Technology Act" of December 2005, pursuant to section 11 cf section 8. The information presented here may be applicable to more than one provision in different appendices, including those not specifically mentioned. We focused our critique to address the information needs under the relevant provisions that relate to our particular area of competence in biotechnology assessment as comprehensively as possible, and lack of commentary on our part towards any information under consideration should not be interpreted as specific endorsement of that information.

Lastly, Codex Alimentarius guidelines allow Norway to ask for specific data of the type we identify and recommend obtaining below. Norway therefore may request this information without concern of a challenge from the World Trade Organisation.

Summary of key findings

After a detailed analysis of many of the portions of the dossier on MON87705 submitted by the Developer, we outline a number of methodological and conceptual weaknesses contained in the dossier, including:

• improper assumptions;

• weakness in study design and/or methodology that bias against the detection of differences;

• lack information on potential relevant adverse effects; and

• improper use of comparators.

These weaknesses seriously undermine any scientifically justified conclusion of safety and therefore should be addressed by requests for new information.

1. The rates, types and pathways of exposure to MON 87705 have not been sufficiently characterized by the Developer. This is essential information to properly characterize risk.

2. The Developer has not addressed several important health issues or substantiated its claims of benefits to be derived from use of MON 87705, including the combinatorial (in food) or cumulative (in environment) effects of both high oleic acid levels and unintended increases and decreases in other fatty acids. This may be of significance for those who suffer from, or are prone to, acute respiratory distress, as elevated fatty acids are associated with the disease or its symptoms and when inhaled can irritate the lungs.

3. Critically, the Developer has not provided a convincing case for having either identified or analyzed off-target effects of the novel dsRNAs expressed in soybean MON 87705, or other unintended metabolic changes.

4. It is significant that the Developer has not excluded the production of novel small peptides being produced by regular but low level expression of intended dsRNAs. The Developer has only argued that they do not exist, and this argument lacks scientific basis. Thus, the molecular characterization is unsatisfactory for concluding that there are no novel protein-based hazards.

5. The standards used for equivalence testing of surrogate proteins in the assessment lack substantive rigor to draw relevant conclusions. The methods and study designs employed by the Developer, in our view, bias against identification of differences.

6. Concerning the social utility of MON 87705 outlined in Appendix 4 Part V, it is highly questionable whether the intended changes in fatty acid profiles caused by the modification is demanded or needed within the Norwegian diet, thus there are no net benefits to Norwegians and merits further attention.

When it comes to considering the benefits to Norwegians of approving MON 87705, we encourage the Direktoratet for naturforvaltning to take a holistic view. The harm of using conventional soybeans in human food comes from overconsumption or substitution of soybean derived oils for oils more fit for purpose. The solution to these problems, particularly exposure to trans fats, is not alteration of the Norwegian food supply by introduction of novel products from overseas agroecosystems, but a commitment to providing good food and social programs that encourage healthy eating.

Summary of recommendations

We propose a number of recommendations, summarized here and detailed in the critique below.

The Direktoratet for naturforvaltning is encouraged to:

1. Rigorously pursue its right to request experimental data from the Developer to answer the questions raised in this submission, before concluding that a rejection would not be justified on the basis of safety.

2. Request from the Developer an exposure analysis that includes consumption by age, sex and ethnic group of Norwegian consumers; and both data on currently consumed amounts of conventional soybean oil and maximum possible consumption of high oleic acid soybean oil, including consumption patterns that might eventuate from new applications of this oil in Norway.

3. Require information from the Developer on the effects of MON 87705 inhalation in animals used as models of acute respiratory syndrome, compared with inhalation of the proper conventional comparator. This should include allergenicity and toxicity analyses.

4. Request a detailed analysis of the structural and functional characteristics and bioactivity of the novel 9,15 isomer likely produced in MON 87705.

5. Request a full chemical compositional description of whole foods prepared under a range of normal cooking and processing conditions using oil derived from MON 87705 and compared to oil from the proper conventional comparator line. This should be followed by animal feeding studies using whole foods produced using these two sources of oil.

6. Request information from the Developer on microbial exposure routes including the effects of oleic acid on environmental flora (particularly those microorganisms that may flow through to human food) and the dietary effects on human flora, particularly the ability of increased exposure to select for resistance and cross-resistance to clinical antibiotics and antiseptics.

7. Request a demonstration the effect from transient expression of the expected RNA hairpin (using a non-integrated construct) or RNAi from transformation with hairpin

RNA alone is the causative agent for the intended phenotype.

8. Request information from the Developer on all RNA molecules unique to MON 87705, or at unique concentrations in MON 87705. Moreover, that information should be informed by appropriate high throughput sequencing methodologies.

9. Request a demonstration that unintended dsRNAs—which may still be unknown—produced in the MON 87705 soybean or secondarily in human cells have no adverse effects.

10. Request that the Developer describe all off-target changes to gene expression in MON 87705, and the potential for the novel RNA molecules (or molecules at novel concentrations), and possible derivatives that may be made in human cells, to cause effects on human cells.

11. Require the Developer to monitor ongoing nucleotide-level changes in the transgene and subsequent changes to the off-target effects of the dsRNA in MON 87705, if it is approved for commercial use. In the absence of such monitoring, approval should be conditional and limited to a period of no more than three years.

12. Request a compositional analysis of MON 87705 be provided from plants grown under intended commercial cultivation methods and compared to its conventional parent when grown under its intended commercial cultivation methods.

13. Request justification and scientific evidence for the criteria chosen by the Developer for inferring biochemical and safety equivalence of *E. coli*- and MON 87705–derived CP4 EPSPS were appropriate.

14. Request data from proper immunostimulation and allergenicity testing of MON 87705 including tests from diet and inhalation exposures. A proper comparator would include the product produced under intended use conditions. Comparisons using immune sera from subjects sensitized to conventional soy are not capable of detecting immune responses unique to MON 87705.

1. Critique of Health Claims and Exposure Information to MON87705

Relevant to Appendix 2 Paragraph C.2; Appendix 4 Parts I – III, V

Health benefits asserted but not scientifically verified or justified

The developer is claiming both industrial and human health benefits of oil derived from MON 87705. The human health benefits are asserted from the general guidance (e.g. discussed in section D.1.C pp. 49-50) for those on a Western diet to avoid certain kinds of fatty acids or fats, more specifically to avoid the foods rich in those kinds of compounds, such as those fried in soybean oil, but not from evidence derived from their product. (For examples of Developer's claims, see Table 1.)

Table 1: Claims of health benefits lack substance or are misleading

Claim	Problem
"The improved nutritional benefit of decreased saturated fatty acids coupled with the expected increased stability makes it well suited for use in bottled vegetable oil, salad dressings, margarine and other similar food products for which commodity soybean oil is used" (p. 17)	No evidence of improved nutritional benefit of oils derived from MON 87705 in any listed product.
"As a result, the reduced saturated fat levels in MON 87705 soybean oil, particularly palmitic acid, can positively impact the goal of limiting dietary saturated fat intake to below 10% of total energy intake" (p. 19)	No evidence that the social goals will be positively impacted. This is pure speculation on the Developer's part.
"Collectively, the scientific literature supports the conclusion that dietary oils rich in oleic acid are a suitable option to provide energy when replacing the use of oils high in saturated fats or TFAs. It is important to note that none of the publications report any incidence of adverse events as a consequence of dietary modifications evaluated in the studies" (p. 232)	While there is considerable evidence of comparative benefit of oleic acid vs. trans fats, there is no evidence that these benefits would accrue from MON 87705. Importantly, it is inappropriate to extrapolate for the absence of evidence of harm of oleic acid in the context of other vectors to the safety of this GM vector of oleic acids. Finally, the risk of inhalation exposure is different for soy derived products than it is for other foods rich in oleic acids.

There is no evidence provided to support the claims listed in Table 1 that MON 87705 will have health benefits for consumers. To our knowledge, none of the studies summarized in Table 42 (pp. 233-234) used GM or conventional soybeans as a vehicle for delivering the oil-manipulated diets, much less the three products that the Developer specifically names. That is, to extrapolate from general findings of the benefits of eating different kinds of oils to the safety of GM soybeans would be like accepting the test of a drug based on the purified compound only and not the final commercial formulation as a pill. This is simply not done, and is not sound science.

In many jurisdictions, claims of specific health benefits would require approval by competent medical and labeling authorities. There are several reasons why these claims (Table 1), based on the evidence provided, are inappropriate at best, and unethical at worst.

1. There are no properly conducted human trials showing that "salad dressings, margarine and similar food products" made with MON 87705-derived oils yield a health benefit.

2. There are no studies, to our knowledge, verifying the general assertion of the benefits of this oil either in isolation or in the context of normal foods.

3. The Developer performed no inhalation studies with animals (only gavage or nutritional feeding studies) and no animal feeding studies substituting the substantially different oil from MON 87705.

4. It is furthermore a methodological mistake for the Developer to make such claims because neither the Direktoratet for naturforvaltning nor the Developer can foresee all kinds of future uses and quantities of exposures to this novel product based on historical uses of a fundamentally different plant—the "conventional" soybean—nor can they anticipate what novel combination of chemicals can result from this new use of soybeans in applications that have traditionally used other plants, such as olives and canola, and their oils. That is, hazard identification is not simply a matter of considering each single significant difference between conventional and this genetically engineered soybean in isolation. Nor is it appropriate to seek other precedents for quantitatively equivalent exposures to oils from different plants or animals. This last point is elaborated immediately below.

Demographic exposure rates and pathways, and future product uses are not sufficiently defined to extrapolate safety or social utility of the product

The developer has provided no information on the demographics of consumption expected in Norway. The Developer says that such information is not available or not available in a usable form (e.g., see p. 211). However, it is not the burden of either the government of Norway or the citizens of Norway to provide this information to the Developer; the burden of demonstrating that this product of modern biotechnology provides social utility and poses no unacceptable risks lies with the Developer.

Moreover, there is significant uncertainty (discussed above) about future uses of the oil, and therefore exposure pathways for consumers. It is Norway's right and regulatory responsibility to both receive such information and to verify its accuracy.

At minimum, the Developer should submit an exposure analysis that includes:

- **consumption by age, sex and ethnic group of Norwegian consumers; and**
- **both data on currently consumed amounts of conventional soybean oil and maximum possible consumption of high oleic acid soybean oil, including consumption patterns that might eventuate from new applications of this oil in Norway.**

This information is important because, firstly, there is no reason to expect that the Developer will only seek to replace conventional soybean oil at current levels of consumption. The Developer may attempt to market high oleic acid soybean oil in place of other conventional high oleic acid oils such as olive and canola. Moreover, with increases in production of MON 87705-derived oil, more solid soy foods might contain MON 87705. In any case, once approved for use the Direktoratet for naturforvaltning will not be able to restrict how the Developer markets their product.

Secondly, we are unaware of any objective and accepted data that average consumption rates are representative of exposure for all consumers across different groups. Some consumers may eat more soybean products than others (e.g., the lactose intolerant, coeliac disease patients, certain ethnic groups) or more fried foods, and thereby soybean oil, than others. For example, Chinese women may consume upwards of 80 times as much soy as Americans or Europeans (Keinan-Boker et al., 2002). It would be in our view inappropriate to use averages to downplay exposure without more thorough documentation of maximum exposures among subgroups in Norway. Maximum exposure analysis is, furthermore, recommended by Codex Alimentarius.

> "Information about the known patterns of use and consumption of a food, and its derivatives should be used to estimate the likely intake of the food derived from the recombinant-DNA plant. The expected intake of the food should be used to assess the nutritional implications of the altered nutrient profile both at customary and maximal levels of consumption. *Basing the estimate on the highest likely consumption provides assurance that the potential for any undesirable nutritional effects will be detected.* Attention should be paid to the particular physiological characteristics and metabolic requirements of specific population groups such as infants, children, pregnant and lactating women, the elderly and those with chronic diseases or compromised immune systems" (emphasis added to p. 19 Codex, 2003).

Thirdly, the Developer clearly expects exposures to increase or they would not be going to the expense of producing a soy oil to replace or supplement existing sources of high oleic acid oils (e.g., olives). These supply-driven increases may also not be experienced uniformly by consumers, dramatically increasing the exposure of some consumers while having little effect on others.

Fourthly, high oleic acid soybean must be "as safe as" conventional soybeans across the spectrum of food uses and normal food preparation practices. If it is not, then any differences may result in currently unanticipated hazards arising from the use of this product in future

contexts, particularly in combination with future genetically modified foods. “As safe as” is critically different as a standard than “safe as” for use as human food. This is especially important for those who may currently, or may in the future, wish to avoid dietary sources of oleic fatty acids for particular health reasons.

Food is a complex mixture of material treated in a way that differs from how animals are exposed to those same materials and therefore must be tested in this complex form, as a whole food under normal conditions. The modification of oil type and quantity will cause oil derived from this soybean to be used in ways, or at quantities, for which there is no history of safe use. According to Codex Alimentarius:

> “The potential effects of food processing, including home preparation, on foods derived from recombinant-DNA plants should also be considered” (p. 18 Codex, 2003).

Fifthly, it is unlikely that the techniques used by the Developer would with confidence have detected all unintended differences in composition (see below). Hence, average soybean use statistics are not suitable for substitution for data that describe the complex of chemicals that may result from heating, particularly repeated heating, of the oil with other metabolite differences at concentrations unique to the use of soybean oils.

Sixthly, the Developer has only considered dietary exposure pathways. Inhalation exposure can be expected to be a significant pathway for many people, and a more direct cause of potential adverse effects. Oleic acid is used to induce acute respiratory distress syndrome in animal models, usually through blood infusion. Humans may more likely have direct, non-dietary exposure to oleic acid through high oleic acid soybeans than other sources of oleic acid. This is because soybean flour is a very common product and thus inhalation of soy flour is more likely than inhalation of meal produced from olives. Edible soybean flour production was estimated at 2 million tons by 1992, up from only 60,000 tons in 1960 (Berk, 1992). It is used in baking, cereals and pasta. It has important uses in replacing wheat flours especially for those with coeliac disease (Berk, 1992).

Inhalation provides direct lung cell exposure to oleic acid, and may more closely mimic infusion. Moreover, inhalation sensitization to allergens can be more important than dietary sensitization.

“[I]it has to be considered that transgenic plants may be used in industrial processing; hence other exposure routes and sensitization scenarios might become important. For example, manufacturing large amounts of transgenic soy containing a food allergen may induce respiratory sensitization due to the generation of allergen-containing dust” (Spok et al., 2005).

We recommend that the Direktoratet for naturforvaltning request information from the Developer on the effects of MON 87705 inhalation in animals that are used as models of acute respiratory syndrome, compared with inhalation of the proper conventional comparator. This should include an analysis of allergenicity and toxicity.

Unsubstantiated and potentially misleading claims based on the Mediterranean diet

As far as we are aware, there is not a single scientific study that shows any of the benefits associated with a "Mediterranean diet" (associated with foods rich in oleic acid) extended to the use of high oleic acid soybeans. In fact, we are unaware of studies that show sustained or actual benefits of high oleic acids in isolation; those benefits are in the context of a whole diet. That fact is obscured in the dossier where the Developer makes repeated reference to the benefits of oleic acids despite the lack of evidence of any benefit from the use of high oleic acid soybeans or their oils. Reducing the benefits of certain diets and lifestyles to particular chemical ingredients such as oleic acid, or in comparison to whole foods or plants that are compositionally different from soybeans, would be a significant methodological mistake.

We cannot emphasize enough that the solution to problems caused by trans fats is not alteration of the Norwegian food supply by overseas agroecosystems, but a commitment to providing good food and social programs to encourage healthy eating in Norway.

There are many potential health benefits from substituting oleic acid for other fatty acids that may form trans fats particularly after being hydrogenated. However, there is at present no substantial scientific claim that in all foods and for all people oleic acid is preferential. By increasing the range of basic food sources that are homogenized for being high oleic acid sources, we simultaneously remove food options from those who may wish to avoid high oleic acid foods, or increase the costs and challenge to consumers wishing to avoid these foods. For example, high oleic acid levels are associated with potential health hazards among those with certain respiratory conditions.

"[O]lives (and thus oleic acid) are important ingredients of the healthy Mediterranean diet. On the other hand, patients with acute respiratory distress syndrome have elevated serum levels of oleic acid, and infusion of oleic acid in animals results in an acute lung injury–type syndrome" (p. 424 Matalon and Ji, 2005).

2. Missing information on potential adverse effects

Relevant to Appendix 2 Paragraph C.2, Paragraph D; Appendix 4 Parts I – III, V and VI

Specific information on the C18:2 isomer 9,15 is essential to a risk assessment

Other GM soybeans modified using dsRNA, as was MON 87705, to achieve higher levels of oleic acid also have altered linoleic acid levels (FSANZ, 2000, FSANZ, 2009). Interestingly, in those cases linoleic acid C18:2 was also decreased overall, but a new isomer (9,15) that appears to be unique to GM soybeans created using dsRNA consistently appears (Kurenbach et al., 2009).

The reaction pathways, and potential to form trans fats, of this isomer are unknown in human foods prepared using soybeans or soybean oil. Since this isomer is consistently found in soybeans derived from dsRNA modification, and not from conventional soybeans with histories of safe use, a specific evaluation of this species is warranted.

While other kinds of food sources may naturally contain the 9,15 isomer (p. 33 FSANZ, 2009b), we counsel against this kind of comparison. Foods such as mango pulp, cheese and

beef are compositionally and nutritionally not comparable to soybean or soybean oil, are not cooked or mixed with the same range of ingredients, and thus are not predictive of the safety or otherwise of soybeans that make high levels of the 9,15 isomer.

The Direktoratet for naturforvaltning should request a detailed analysis of the structural and functional characteristics and bioactivity of the novel 9,15 isomer likely produced in MON87705.

Effects of heating

High oleic acid soybeans are being proposed for use as human food and may in particular be used in high temperature applications (regardless if this is the intent of the Developer). However, we could find no safety studies on the chemical composition of the oil after heating, feeding studies using products fried in the oil, or solid soy food products derived from MON 87705. Given that high oleic acid soybean oil is chemically different from oils derived from conventional soybeans, oilseed rape, sunflower, safflower and olives, how has the Developer determined that there are no hazards from this novel food?

There is evidence of adverse effects of cooking high oleic acid GM soybeans , also created through silencing of the *fad2* gene (FSANZ, 2000). Food Standards Australia New Zealand (FSANZ) summarized two separate feeding studies, one involving pigs and the other chickens, in which the processing of GM high oleic acid soybean oil at cooking temperatures ranging from 80-105ºC reduced its nutritive value (see Tables 13 and 14 of A387). However, we cannot tell from these data whether the effects were anti-nutritive or toxic. Nevertheless, those studies provided an "indication of how much food (in pounds) it takes to put on 1 lb of body weight in the animal" (p. 37 FSANZ, 2000) and revealed that animals fed on heated GM high oleic acid soybeans often were less able to convert food energy into body mass. When pigs or chickens were fed the GM high oleic acid soybeans, the efficiency of feed conversion fell in comparison to control diets.

While the Developer also used toasted meal, they did not reveal the temperature of the toasting. This is important because the previous studies discussed also showed that the adverse effect was more pronounced at particular temperatures.

FSANZ offered no explanation for the effect on pigs. At the time FSANZ attributed the effect on chickens to lower amino acid levels in the test diets. The key point here is that the cause of the processing effect on high oleic acid soybeans was never determined. We find no evidence of a processing experiment on soybean MON 87705 to prove that it would not cause the same potential adverse effects as other GM soybeans that have high oleic acid.

We recommend that the Direktoratet for naturforvaltning exercise their option under Codex Alimentarius to request a full chemical compositional description of whole foods prepared under a range of normal cooking and processing conditions using soy meal or oil derived from MON 87705 and compared to meal and oil from the proper conventional comparator line. This should be followed by animal feeding studies using whole foods produced using this GM soybean MON 87705 and its closely related

conventional parent.

Oleic acid and microflora

Oleic acid has antibacterial and anti-viral properties (Thormar et al., 1987, Zheng et al., 2005). These properties may result in a change in flora that use soybeans as a habitat, and may increase quantities of oleic acid in human food as this source of oil becomes adopted for food preparation. New combinations of food may be exposed to a soybean source of high oleic acid, which might also then quantitatively increase pressure on food-borne microorganisms to acquire resistance to oleic acid. The resistance to oleic acid should be evaluated for the possibility that it may confer cross-resistance to clinical antibiotics or antiseptics.

We recommend that the Direktoratet for naturforvaltning request information from the Developer on the effects of oleic acid on environmental flora that may flow through to human food and the dietary effects on human flora, particularly the ability of increased exposure to select for resistance and cross-resistance to clinical antibiotics and antiseptics.

3. Unintended potential adverse effects derived from the intended modification for dsRNA-mediated silencing

Relevant to Appendix 2 Paragraph C.2, Paragraph D; Appendix 4 Parts I – III, V

Lack of understanding of core mode of action limits potential to ascertain unintended effects

The modification of MON 87705 is based on dsRNA silencing, a type of manipulation that has not benefited from human food safety studies to our knowledge (Heinemann, 2009). To emphasize the uncertainty such methods bring to hazard identification and thus risk assessment we quote the food safety regulator FSANZ where they say about a product similar to MON 87705: "The Applicant speculates that suppression of expression of the endogenous *gm-fad2-1* gene is mediated via co-suppression in which the introduced fragment leads to an overabundant production of sense mRNA which in turn leads to production of dsRNA *via a pathway that is still not understood*" (emphasis added to p. 12 FSANZ, 2009). Under such circumstances where the biochemistry of the modification itself is considered to be *speculation and is not understood*, it is difficult to have confidence that the Developer could report all unintended effects of the modification.

dsRNA is not GRAS (generally regarded as safe)

Research by the Developer has shown that novel dsRNA molecules at unique concentrations in transgenic plants can transfer through food to animals wherein these molecules or derivatives of these molecules cause adverse effects in insects (Baum et al., 2007):

> *[…] we demonstrate that ingestion of doublestranded (ds)RNAs supplied in an artificial diet triggers RNA interference in several coleopteran species […]. This may result in larval stunting and mortality.*

Researchers demonstrated that dsRNA can be infectiously transferred through food to gut cells in insects, and subsequently spread within the animals (Gordon and Waterhouse, 2007). The dsRNA created in the transgenic dsRNA-insecticide plants described were in fact derivative or "secondary" RNA species, and notably Baum et al. (2007) are sure that they were the cause of more derivative RNA molecules after processing by the RNAi activity in the target insects. We encourage the Direktoratet for naturforvaltning to seek evidence that secondary processing in human cells of novel dsRNA molecules created by MON 87705 would not generate any biologically active dsRNAs in human cells, or in the cells of particular wildlife species.

The Developer makes the erroneous claim that:

"The same evidence holds true for RNA. Suppression of FATB1-A and FAD2-1A soybean genes in MON 87705 is mediated by double stranded RNA (dsRNA) molecules. Double stranded RNAs are commonly used by eukaryotes, including plants, for endogenous gene suppression and pose no novel risks from a food, feed or environmental perspective. The nucleic acids that comprise RNA have a long history of safe consumption and, as mentioned previously, are considered GRAS by the US Food and Drug Administration (FDA, 1992)" (p. 35).

The assertion is incorrect in saying that because dsRNAs are in food, our diets include exposure to *the* dsRNAs produced in, or because of exposure to, MON 87705. The effects of dsRNA are sequence-specific and the Developer has provided no evidence that the transgenic dsRNA has ever been consumed by humans (or wild vertebrate and invertebrate animals) or consumed in prepared foods. [Note as well that RNAi is not a physical molecule, it is an observed phenotype. dsRNA is the molecule that is consumed.]

That is to say, a history of consuming small RNA molecules in plants is not the same as extrapolating the safety of all small RNA molecules, any more than a history of consuming proteins attests to the safety of every protein. When a small RNA molecule will or might not act as a gene regulator is not always known in advance. Therefore, it cannot be assumed that novel small RNAs that might be created in high oleic acid soybeans will likewise be safe. Certainly, the dsRNA used as an insecticide is not safe from the perspective of pest insects targeted in other work described above (Auer and Frederick, 2009, Baum et al., 2007) and by extrapolation some small RNAs may not be safe for humans. Indeed, the plants that humans traditionally consume may be precisely those that produce small RNAs that have not been toxic to us.

The assertion is also misleading because it does not take into account inhalation exposure, only dietary exposure.

From the above, it is clear that dsRNA can have significant biological impact. Recent research (Baum et al., 2007, Gordon and Waterhouse, 2007, Mao et al., 2007) establishes beyond

doubt that novel RNAs of recombinant or synthetic origin cannot be "generally regarded as safe" but must be tested and demonstrated to be safe when consumers or wildlife is exposed through food or inhalation.

Claims of specificity are misleading

The Developer makes other misleading claims about the predictable effects of dsRNA: "Because of its high specificity and efficacy, the RNAi pathway can be used to control the expression of a gene to attain a specific phenotype" (p. 41-42).

Contrary to the implication that dsRNA is specific; it can be the source of many unintended effects. dsRNA molecules generate many off-target effects.

The genes silenced by dsRNAs are specific to the dsRNA, rather than dsRNAs are specific to target genes (Jackson et al., 2003). Sometimes hundreds of off-target transcripts are reduced or silenced (Jackson et al., 2003, Jackson et al., 2006, Ma et al., 2006). For example, Semizarow et al. found that a set of 5 different dsRNA molecules that silence the same gene (AKT1) collectively silenced 840 genes (Semizarov et al., 2003). Species-specific differences in RNA editing further contribute to unanticipated dsRNA species and off-target effects (O'Connell and Keegan, 2006).

Therefore, the transcriptome of the GM crop should be evaluated for all novel dsRNAs. The insecticide findings (above) and the real possibility of off-target effects provide a powerful argument for those companies and regulators who have previously dismissed the need for proper profiling of the transcriptome and proteome in human health and environment safety assessments of GM crops to now accept the importance of such enquiry (Heinemann, 2007).

Furthermore, off-target effects sometimes only change protein levels and not transcript levels (Jackson and Linsley, 2004, Scacheri et al., 2004), making it even more complicated to track effects. Therefore, both the transcriptome and proteome of the GM crop should be profiled for unintended effects.

"[F]urther research into off-target effects [of dsRNA] should be encouraged because the current lack of information creates uncertainties about this particular hazard" (p. 649 Auer and Frederick).

The Developer also incorrectly claims that: "Gene suppression via RNAi requires the transcript from an inserted gene cassette to form a double stranded hairpin RNA, triggering the RNAi pathway and silencing a target sequence through a sequence specific mechanism" (p. 42). Contrary to this characterization of the phenomenon of RNAi, a hairpin, formed by intramolecular base-pairing, *can be* sufficient to cause RNAi, but is not the only way that siRNAs may form. The insertion may result in the production of RNAs that form linear dsRNAs by intermolecular base-pairing with other transcripts. These other dsRNAs may be processed into different active siRNAs than would be predicted from hairpins.

The Developer also claims that "[t]he assembled gene transcript has an inverted repeat that produces dsRNA that, via RNA-based suppression (Siomi and Siomi, 2009), suppresses endogenous FATB and FAD2 genes, thereby producing the desired fatty acid profile of decreased saturated (16:0 palmitic acid and 18:0 stearic acid), increased oleic and decreased

linoleic fatty acid composition in the oil" (p. 42). We note with concern that the Developer has provided *no evidence* that this is the pathway by which the insert has achieved silencing. This is critical, because dsRNA molecules that may form through **inter**molecular base pairing can also cause RNAi, but may have significantly different off-target effects. While the use of the Siomi and Siomi reference in the sentence contained in the dossier and quoted above may give the impression that this publication provides insight into MON 87705, it does not. It is a review on the general phenomenon of RNAi.

To demonstrate the plausibility of the hypothesis that the expected hairpin is the causative agent of RNAi in MON 87705, its physical presence in the soybean should be demonstrated. To satisfy causation, we recommend that the Developer should demonstrate the effect from transient expression of the hairpin (using a non-integrated construct) or RNAi from transformation with hairpin RNA alone. Short of such demonstrations, it is irresponsible to conclude that the causative, or all causative, dsRNA species are known and, by extension, that all off-target effects can be dismissed.

Since in this case the concern is unintended effects on genes that were not targeted by the modification, only microarray or preferably high throughput sequencing techniques would be suitable for proper molecular characterization.

High-throughput sequencing proved to be a powerful and quantitative method to sample transcriptomes deeply at maximal resolution. In contrast to hybridization, sequencing showed little, if any, background noise and was sensitive enough to detect widespread transcription in >90% of the genome, including traces of RNAs that were not robustly transcribed or [were] rapidly degraded (p. 1239 Wilhelm et al., 2008).

Additionally, researchers have applied this technique to organisms at different stages of their life cycles and under different environmental conditions, demonstrating that this technique can be effectively used to describe the transcriptome of different tissues, stages of development and at different times (Wilhelm et al., 2008). It can be used on any kind of GMO (Lu et al., 2007).

Not only has full transcriptome profiling become possible, it is also seen as "necessary to sample the full complexity of small RNAs in plants and likely other organisms as well. Application of this method to several key mutants affecting small RNA biogenesis pathways can quickly lead to the identification of candidate miRNAs, trans-acting siRNAs and other interesting classes of small RNAs" (p. 116 Lu et al., 2007). The sequencing technique is less prone than global microarrays to ambiguities due to background detections (Kristensen et al., 2005, Wilhelm et al., 2008).

Codex Alimentarius allows the Direktoratet for naturforvaltning to ask for information on RNA molecules without concern of action from the WTO.

"Information should be provided on any expressed substances in the recombinant-DNA plant [or microorganism]; this should include: A) the gene product(s) (e.g. a protein or an untranslated RNA)…E) where possible, the amount of the target gene product(s) if the function of the expressed sequence(s)/gene(s) is to alter the accumulation of a specific endogenous mRNA or protein" (p. 14 and 39 Codex,, 2003).

We recommend that the Direktoratet for naturforvaltning request information from the Developer on all RNA molecules unique to MON 87705, or at unique concentrations in MON 87705, all off-target changes to gene expression in MON 87705, and the potential for the novel molecules (or molecules at novel concentrations), and possible derivatives that may be made in human cells, to cause effects on human cells. Moreover, that information should be informed by appropriate high throughput sequencing methodologies.

Mutation potential for genes that encode small RNAs

Finally, there is evidence that "[m]utation rates in genes for small RNAs can be high relative to protein-coding genes" (p. 648 Auer and Frederick, 2009). Thus, approval of GMOs that rely on small RNA molecules for their effects may not be suitable for a single approval regulatory system because changes in these sequences over time can lead to further and unanticipated off-target effects.

We recommend that the Direktoratet for naturforvaltning should indicate how they or the Developer will monitor ongoing nucleotide-level changes in the transgene and subsequent changes to the off-target effects of the dsRNA. In the absence of such monitoring, approval should be conditional and limited to a period of no more than three years.

Potential adverse effects from unanticipated novel proteins

The Developer has come to conclusions that overstep the evidence.

For example:

"There are no new constituents present in MON 87705 and therefore, no further testing is required" (p. 232).

"It should be noted that production of a protein from a double stranded RNA is highly unlikely due to the structure inhibiting ribosomal translational scanning (Kozak, 1989), thus the potential for RNAi introduced protein toxicity or allergenicity is highly unlikely. RNAi originating from biotechnology-derived crops do not differ from the RNAi already present in food, and therefore have a history of safe consumption. Consequently, the use of RNAi in plant biotechnology is a precise and safe tool" (p. 42)

"The FAD2-1A/FATB1-A suppression cassette encodes for dsRNA, and it is extremely unlikely to code for a protein. Therefore, CP4 EPSPS is the only newly expressed protein in MON 87705" (p. 76).

These assertions are both misleading and incorrect. That is, it is misleading to suggest that the inhibition of translation of dsRNAs can be dismissed as unlikely and, by extension, to assert without proper evidence that there are no new proteins or metabolic constituents in MON 87705. The inhibition of translation by formation of dsRNA alone is the weakest

manifestation of RNAi precisely because it is more likely to result in translation than when the target mRNA is hydrolytically cleaved. The production of protein instead may be *very likely* but at lower levels. The only way to be certain that MON 87705 is not producing a protein with either toxic or immunomodulating characteristics is to test it for such proteins, beginning with synthesizing the peptide produced by translation of the hairpin RNAs.

Further, the Developer did not include for comparison purposes the compositional analysis of MON87705 that would result from intended product use, i.e. in conjunction with glyphosate application. There is evidence that the intended cultivation practices may themselves have significant effects on composition (Ozturk et al., 2008). Therefore MON 87705 treated with Roundup or other glyphosate-based herbicides should be compared to its proper conventional parent grown under commercially relevant conditions.

We recommend that a compositional analysis of MON 87705 be provided from plants grown under intended commercial cultivation methods and compared to its conventional parent when grown under its intended commercial cultivation methods. Moreover, MON 87705 should be tested for immunostimulation and allergenicity effects including tests from diet and inhalation exposures that include the product produced under intended use conditions.

4. Detecting differences: Establishment of bioequivalence between in planta and *E. coli* produced CP4 EPSPS

Relevant to Appendix 2 Paragraph C.2 and Paragraph D; Appendix 4 Parts I – III

The criteria used by the Developer is unacceptably weak to establish bioequivalence

Included in the dossier is a study of the "bioequivalence" of surrogate transgenic CP4 EPSPS protein derived from the bacterium *E. coli*, in the place of the transgenic protein actually produced in plants (and put into the environment/consumed by other organisms).
The Developer defines four criteria for equivalence of in planta and *E. coli* produced CP4 EPSPS:

1. *Immunoreactivity with CP4-specific antibodies: the immunoreactive signal of the test protein should be within ± 30% of the reference protein.*
2. *Molecular weight: the apparent molecular weight, by SDS-PAGE, of the test protein should be within ± 10% of the reference protein.*
3. *Activity assay: the functional activity of the test protein should be within ± 50% of the reference protein.*
4. *Glycosylation status: both test and reference proteins are not glycosylated.*

(p. 21 of Wang et al.,2009)

Small changes in primary and secondary protein structure can impart important changes in their bioactivity (Haider and Ellar, 1989; Geiser et al., 1996, Walsh and Jeffries, 2007). Therefore, the above criteria are permissive of possible small structural but significant

functional changes in bioactivity or immunogenicity, and represent an extremely weak standard of safety. *Additionally, no rational is given for each of the particular quantitative values, which are not to our knowledge based on scientific evidence.*

Assays used to establish bioequivalence by the Developer are inappropriate for detecting differences

First, the antigen used to raise anti-CP4 EPSPS antibody/antibodies, and the antibodies themselves utilized in the immunoreactivity assay lacks description, e.g. whether the antigen was derived from an *E. coli* expression system in the former or if it is a monoclonal or polyclonal antibody in the latter. It is impossible to say, using the evidence provided, that the antibodies would in fact detect all isoforms of recombinant-CP4 EPSPS that might be produced in-planta, were they present in the sample. In our view, a precautionary regulator should conclude that the Developer has profiled only a single epitope on an unglycosylated recombinant- CP4 EPSPS isoform. This brings into question the subsequent inference of equivalence and use of *E. coli*-derived CP4 EPSPS as a surrogate for MON 87705-derived CP4 EPSPS.

Second, the Developer's means of determining glycosylation status of the two proteins via hybridization of glycoproteins to probes is not the ideal method for sensitive detection of protein glycosylation. A more complete profile is possible using oligosaccharide mapping, liquid chromatography, and mass spectrometry (Werner et al, 2007).

Third, for MALDI-TOF spectrometry, "only experimental masses that matched expected masses are listed" by the Developer (p. 33 Wang et al., 2009). MALDI-TOF was used as a means to identify the protein, and coverage was about 80% of the amino acids in the protein. From the data presented it is not clear if fragments were detected that did not match the expected sizes. These could hint at unexpected modifications after translation. Furthermore, the coverage sufficient to establish the identity of a protein is not necessarily sufficient to exclude post-translational modifications 100% coverage is necessary to establish equivalence.

> *If the aim is to positively identify a protein, it is usually sufficient to detect 50-60% of the amino acids in the protein sample and compare them to a database or the calculated expected results. On the other hand, if the aim is to establish that a protein is identical and has not been altered (e.g. by PTM), then complete coverage is necessary […]. Moreover, MS can fail to distinguish between, for example, two monosaccharide PTMs of identical molecular weight (Küster, B. et al., 2001).*(https://bat.genok.org/bat/?sp=html/topic_guides/ch3_insert_to_trait/proteome_and_metabolome/proteome_testing/ms.html)

In summary, the standard set by the Developer for bioequivalence is unacceptably low and not justifiable, based on current scientific knowledge for the use of *E. coli*-produced CP4 EPSPS protein in studies to assess the safety of CP4 EPSPS protein present in MON 87705 soybean.

We recommend that the Direktoratet for naturforvaltning seek scientific evidence to confirm that the criteria chosen by the Developer were appropriate for inferring

biochemical and safety equivalence of E. coli- and MON87705–derived CP4 EPSPS.

Comparisons using immune sera from subjects sensitized to conventional soy are not capable of detecting immune responses unique to MON87705

In section 7.9.2, "Assessment of allergenicity of the whole GM plant or crop" a quantitative ELISA assessment of human IgE binding to MON 87705 soybean, control and reference soybean extracts were performed.

The Developer submitted the results of an allergenicity test in which the sera from "soybean allergic patients" was incubated with protein extracts prepared from the MON 87705 seed, control soybean, and 17 "conventional soybean varieties" and then was analyzed by enzyme linked immunosorbent assay (ELISA). Focusing on the similarity of reaction profiles, the Developer concluded that, based on the levels of endogenous soybean allergens, the allergenic potential of MON 87705 is unchanged from conventional soybean.

Based on our understanding of the experimental design, the study used sera from people sensitized to conventional soybean, not soybeans with elevated oleic acid composition. These individuals would not have mounted an immune reaction to an unknown allergen unique to high oleic acid soybean MON 87705. Therefore the study only provides baseline data about the generic allergenicity of soybeans; it is not capable of distinguishing the allergenic potential of MON 87705 from conventional soybean for people never exposed to MON 87705. We fail to understand the relevance of this study for demonstrating the safety of MON 87705. Moreover, the study was limited to 13 soy-sensitive individuals with unknown histories of sensitization. People could be exposed to MON 87705 both in the diet and through inhalation of flour. Therefore, the study should include an assessment of the allergenic potential of MON 87705 through both dietary and inhalation sensitization.

We recommend that the Direktoratet for naturforvaltning requests data from proper immunostimulation and allergenicity testing of MON87705 including tests from diet and inhalation exposures.

Conclusion

In on our detailed analysis of the dossier submitted by the Developer concerning MON 87705, a number of improper assumptions, lack of information, weakness in study design or methodological treatment that bias against the detection of unintended differences, missing or deficient information on potential adverse effects were identified, all of which do not merit the conclusion of safety given by the Developer.

The Developer has not addressed several important health issues with MON 87705,nor provided compelling evidence that off-target effects of the novel dsRNAs expressed in soybean MON 87705, or other unintended metabolic changes, do not occur. It is significant

that the Developer has not excluded the possible regular but low level expression of small peptides coming from intended dsRNAs. The Developer has only argued that they do not exist, and this argument lacks scientific basis. Thus, the molecular characterization is unsatisfactory for concluding that there are no novel protein-based hazards.

Concerning the social utility of MON 87705 outlined in Appendix 4 Part V, it is highly questionable whether the intended changes in the fatty acid profile of MON 87705 is demanded or needed within the Norwegian diet, and merits further attention.

Lastly, we wish the regulator to consider whether the solution to these problems, particularly exposure to trans fats, is not alteration of the Norwegian food supply by introduction of novel products from overseas agroecosystems, but a commitment to providing good food and social programs that encourage healthy eating.

Please refer to the "Summary of key findings" and "Summary of recommendations" at the beginning of this document for specific recommendations to improve the risk assessment of MON 87705.

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