Maisons-Alfort, 19 October 2012

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

concerning an analysis of the study by Séralini *et al.* (2012) "Long term toxicity of a ROUNDUP herbicide and a ROUNDUP-tolerant genetically modified maize"

ANSES undertakes independent and pluralistic scientific expert assessments.
ANSES primarily ensures environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

It provides the competent authorities with all necessary information concerning these risks as well as the requisite expertise and scientific and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).

Its opinions are made public.

On 24 September 2012, ANSES received a formal request from the Minister for Social Affairs and Health, the Minister of Ecology, Sustainable Development and Energy, the Minister of Agriculture, Food and Forestry, and the Minister for the Social Economy, Solidarity and Consumer Affairs attached to the Ministry of Economics and Finance, to issue an Opinion based on an analysis of the recently published study "Long term toxicity of a ROUNDUP herbicide and a ROUNDUP-tolerant genetically modified maize" (Séralini *et al.* 2012).

1. BACKGROUND AND PURPOSE OF THE REQUEST

On 19 September 2012, the journal Food and Chemical Toxicology published a study by Séralini *et al.* on the long-term toxicity of ROUNDUP and NK603 maize, which is glyphosate-tolerant. In particular, the authors noted an increase in mortality and the incidence of tumours in several groups of rats fed for two years on NK603 glyphosate-tolerant maize, whether or not the maize had been treated with ROUNDUP WEATHER MAX or given drinking water containing various doses of the herbicide ROUNDUP GT PLUS.

ANSES was immediately informed of a future request with the aim of:

- establishing whether or not this publication casts doubt on the conclusions of previous assessments of this genetically-modified organism (GMO) or of the herbicide ROUNDUP, and in particular whether it can be considered conclusive with regard to the possible health risk of food from plants containing the NK603 event,
- assessing from this whether the experimental protocol and the conclusions of this study cast doubt on current or future guidelines for the assessment of health risks.

At the same time, the French Government also asked the French High Council for Biotechnologies (HCB) to investigate the aspects specifically related to NK603 maize.

On the same day this paper was published in the journal Food and Chemical Toxicology, it was the subject of comment in an article in the French weekly *Nouvel Observateur*. The study has attracted considerable attention from the media, reviving the intense public debate on the issue of GMOs and plant protection products.

A number of scientists or scientific groups rapidly expressed opinions in the written press or *via* the Internet and, in many cases, cast doubt on the scientific value of the study, while others highlighted the relevance of the questions raised, the innovative nature of the study and its intrinsic qualities.

Moreover, several Agencies in Member States of the European Union and the European Food Safety Authority (EFSA) also received requests and have issued opinions restricted to a scientific analysis of the study, especially EFSA, Germany's BfR[1], the Dutch RIVM[2] and the Danish DTU[3]. These bodies found that the author's conclusions are insufficiently supported by experimental evidence as a result of the study's inadequate protocol, presentation and interpretation. They also call for the authors to publish the full data on which the study was based. The BfR does underline, however, that the results of this study can be seen as a contribution to the experimental study of the possible influence of co-formulants on the long-term effects of plant protection products.

The documents sent to ANSES by Monsanto express similar criticisms.

It is therefore in a context of intense debate, with a short timeframe and after the issuing of opinions by a considerable number of scientists and collective expert assessment agencies, that ANSES now offers its own analysis of the work described in the paper by Séralini *et al*. (2012) and also gives an opinion on the relevance of reconsidering the way in which the health risks attached to GMOs and plant protection products are assessed.

2. ORGANISATION OF THE EXPERT ASSESSMENT

This expert appraisal was carried out in accordance with the French standard NF X 50-110 "Quality in Expertise - General Requirements of Competence for Expert Appraisals (May 2003)".

ANSES set up an emergency collective expert assessment group (the "NK603 R" ECEAG), made up of experts from a range of disciplines. Its composition (Annex 1) and the experts' public declarations of interest will be made public at the same time as this Opinion. The ECEAG held meetings on 28 September and 3 and 15 October and validated its assessment report electronically on 19 October 2012. ANSES also had several discussions with the HCB and a meeting with representatives of the ECEAG took place on 17 October.

ANSES participated in several meetings for the exchange of information organised by EFSA, on 28 September and 11 and 18 October.

ANSES held two hearings on 10 October 2012, the minutes of which have been validated by the interviewees and are being published at the same time as this Opinion. These concern:

- Several of the co-authors of the study (Messrs Séralini, Spiroux de Vendômois, Defarge, Gress and Mesnage), who were asked to present the results, answer certain questions from ANSES, and give their opinions on current methods for assessing the risks to health of GMOs and plant protection products.
- François Veillerette, President of the "Générations Futures" association, who was asked to express his association's view on current methods for assessing the risks to health of GMOs and plant protection products.

Monsanto was also invited, but in view of the short timeframe preferred to send information in written form on 17 October, which is also being published simultaneously with this opinion.

After the hearing, Mr Séralini sent ANSES, on 15 October, raw data on the mortality of the experimental animals (the data corresponding to Figure 1 in the article by Séralini *et al.*, 2012) and

[1] Bundesinstitut für Risikobewertung
[2] National Institute for Public Health and the Environment
[3] Danmarks Tekniske Universitet

the onset of non-regressive tumours (data corresponding to Figure 2 in the same article), without distinguishing the nature of the tumours.

3. ANALYSIS AND CONCLUSIONS OF THE EMERGENCY COLLECTIVE EXPERT ASSESSMENT GROUP (ECEAG)

In order to understand the context in which the paper by Séralini et *al*. (2012) was published, it is necessary to:

- recall the regulatory context and the general scientific principles underpinning the authorisation process for GMOs and plant protection products,
- list the scientific publications relevant to this Opinion so as to be able to undertake a critical review of the main publications directly related to the issues raised.

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3.1 Regulatory context for the authorisation of genetically-modified organisms and plant protection substances

3.1.1. Expert assessment process and requirements for the authorisation of GMOs

3.1.1.1 Expert assessment process and authorisation of GMOs

Genetically-modified organisms (GMOs) are defined as organisms or microorganisms in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination (Article 2 of Directive 2001/18/EC).

Using genetic engineering techniques, it is possible to transfer selected genes from one organism to another, sometimes between different species. This technique enables the introduction into the genetic make-up of the modified organism of one or more genes coding for proteins conferring new properties. Any sequence of DNA inserted into another organism is called a "transgene". Transferred genes can come from a wide range of living organisms as a result of the universal nature of the genetic code. This technique can be applied to microorganisms, plants or animals. To date, applications for which marketing authorisations (MAs) have been requested for use in food or feed mainly concern genetically-modified plants (GMPs).

Since 2003[4], there has been a specific European regulatory framework governing the use of GMOs (Regulation EC 1829/2003[5]). This Regulation defines, for GMOs (animals, plants, microorganisms, etc.), methods for assessing the risk to food and feed and to the environment, and entrusts this assessment to the European Food Safety Authority (EFSA).
However, as concerns GMOs, EFSA enables the competent authorities of each of the Member States to assess the dossiers and to provide their comments. In France, the competent authorities are the Ministry for the Social Economy, Solidarity and Consumer Affairs attached to the Ministry of Economics and Finance and the Ministry of Agriculture, Food and Forestry. These authorities requested that ANSES investigate the risk to humans and animals of food and feed containing GMOs and also that the HCB (French High Council for Biotechnologies) investigate more specifically the environmental risks.
The procedure followed by ANSES involves an examination of the data provided, verification of their scientific validity and their compliance with regulatory requirements, and an assessment of the health risks in view of the information submitted. When the procedure is complete, conducted according to the collective expert assessment principles applied by ANSES, the Agency's conclusions are presented in an Opinion. This Opinion guides the French vote during the authorisation procedure at the European Commission.

In 55% of the cases examined concerning genetically-modified plants, the Agency deemed that the applicant had submitted insufficient data to enable a conclusion to be drawn on the health issues related to consumption of the GMO in question, and in these cases additional comments were submitted to EFSA.

3.1.1.2 Guidelines for the assessment of GMOs

In 2002, AFSSA drew up guidelines for assessing the safety of GMOs in respect of their use in food and feed (AFSSA 2002). When Regulation EC 1829/2003 was adopted, EFSA developed guidelines detailing the requirements of this Regulation. According to these guidelines (EFSA 2006; EFSA 2009b; EFSA 2011b), the application dossier must include a series of studies designed to identify and characterise any harmful effects related to the consumption of genetically-modified plants or products derived from them, by humans or animals.
The guidelines lay out the information to be provided by the company that must be included in the application for a GMO marketing authorisation in Europe.

[4] Before 2003, applications for use in "human foodstuffs" were governed by Regulation EC No.258/97, known as the "Novel Foods Regulation". This Regulation required that foodstuffs not traditionally consumed in Europe before 1997 (including GMOs), undergo risk assessment prior to authorisation. The use of GMOs in animal feed was regulated by Directive 2001/18.
[5] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically-modified food and feed.
http://ec.europa.eu/food/food/animalnutrition/labelling/Reg_1829_2003_en.pdf

3.1.1.2.1: Analytical data

Molecular characterisation

An initial set of data are used for the molecular characterisation of the GMP. The application dossier must therefore include:

- The DNA sequence of the transgene introduced into the plant to verify that it corresponds fully with the sequences described in the vector used for transgenesis,
- The results of analysis for describing the newly expressed proteins in the GMP,
- Characterisation of the genomic regions flanking the transgene () to verify that the insertion has not occurred in a coding sequence, and to assess the possible production of chimeric proteins or peptides,
- Bioinformatics analyses to search for homologies between the proteins produced by the transgene, the peptides that can be produced in the regions flanking the insert and the proteins or peptides known to be toxic or allergenic,
- The results concerning the genetic stability of the insert in the plant over successive generations.

Comparative analysis of the GM plant and the comparator

The application dossier should also include agronomic and chemical composition data to enable comparison of the GM plant with its closest control (near-isogenic line) (EFSA 2011a) on the basis of its agronomic and phenotypic properties and its chemical composition. The chemical composition analysis concerns several dozen principal compounds (amino acids, fatty acids, etc.), compounds with important nutritional properties such as vitamins, and certain metabolites and antinutritional factors. The nature of the latter depends on the plant species and is laid down in specific OECD[6] guidance documents[7] for each species.

The samples studied come from plants cultivated in fields at several different sites and sometimes over several seasons to take different environmental conditions into account. The experimental design must rigorously follow EFSA's recommendations in terms of repetitions and numbers of sites and must include non-transgenic commercial varieties of the same species. The analysis of the data must follow precise statistical methods (EFSA 2009a). In conclusion, the analysis of these results shows whether or not the GM plant and the non-GM control have the same composition regarding the analysed compounds.

3.1.1.2.2: Toxicity, food-grade and allergenicity studies

Toxicity studies

The assessment of potential toxicity of the GMP and the absence of harmful effects on human and animal health is an essential step in assessing the application. It depends on toxicological studies using laboratory animals (mostly rodents). These are internationally-recognised standard toxicity tests (OECD, Annex 2) conducted under conditions that comply with good laboratory practice (GLP) (Annex 2) for which the applicant must also provide the full study report.

The following animal tests must be conducted:

- An acute toxicity study by single administration of the protein produced by the transgene to several groups of mice which are then examined for 14 days. In the case of new proteins, these are re-administered to the mice for 28 days (EFSA 2011b)[8],
- A repeated–dose 90-day oral toxicity study in rodents on whole food/feed by administration of a part of the plant. The results in terms of growth, consumption and haematological, biochemical and urinary parameters are compared between the groups of animals having consumed the GMP and the groups of animals having received the control plant.

[6] OECD: Organisation for Economic Cooperation and Development

[7] Consensus Documents for the Work on the Safety of Novel Foods and Feeds
http://www.oecd.org/science/biosafety-biotrack/consensusdocumentsfortheworkonthesafetyofnovelfoodsandfeeds.htm

[8] It should be pointed out that these tests, which require a large quantity of proteins, are often carried out with a protein coded for by the same gene but produced in a bacterium. A series of studies demonstrating the equivalence between the protein tested and the protein expressed in the GMP is provided in the application dossier.

This 90-day oral toxicity study on whole food/feed in rodents was not systematically mandatory in the EFSA guidelines, but was decided on a case-by-case basis. In France, ANSES will not rule on applications concerning primary genetic transformation events without this test. The European Commission is currently in the process of making these studies mandatory as part of the process to consolidate the guidelines that appeared as an annex to Regulation EC 1829/2003.

At present, toxicological studies targeting reproduction and development functions (reproduction, development, teratogenicity, etc.) are not mandatory. They can however be requested depending on the potential exposure, the nature and quantitative significance of the differences in chemical composition observed between the GMO and its non-GMO control, or the results of the nutritional assessment and 90-day oral toxicity study on whole food/feed.

Nutritional studies of genetically modified feed

In order to demonstrate that products intended for animal feed have equivalent nutritional qualities, the chemical composition analysis is often supplemented with a nutritional study. The purpose is to verify that groups of animals given feed from a genetically-modified plant show the same zootechnical characteristics (in terms of growth, weight, state of health, etc.) as groups receiving feed from isogenic plants and commercial varieties of the same plant. These tests are often carried out on chickens, over a period covering the usual economic lifetime of broilers (42 days).

This part of the application dossier can also include other elements from *in vitro* or bioinformatic analysis:

- *In vitro* digestibility tests simulating intestinal or gastric digestion in humans or animals and verifying that the resulting proteins and peptides are broken down during the digestion process,
- Physico-chemical properties of the protein(s) produced by the transgene,
- Bioinformatic analysis comparing sequences of the protein(s) produced by the transgene with sequences of the proteins and peptides catalogued as toxic in public databases.

Assessment of potential allergenicity

EFSA recently published a detailed Opinion following its assessment of the allergenicity of GMOs intended for food or feed (EFSA 2010). The new issues raised in this Opinion were incorporated in the revised guidelines (EFSA 2011b). Therefore, the revised guidelines include:

- an assessment of the allergenicity of the newly-expressed protein(s), including the origin of the gene, the structural, biological and physio-chemical characteristics, a comparison of the homology of the amino acid sequence between the newly expressed proteins and known allergens, and *in vitro* tests for resistance to pepsin, and digestibility;
- an assessment of the food or feed's allergenicity involving the whole GMP with, if necessary, an analysis of any possible over-expression of natural endogenous allergens.

3.1.1.3 The Agency's contribution to changes in the guidelines

Like other EU Member State Agencies and jointly with EFSA, ANSES contributes in drawing up and modifying the guideline documents for use by industrial applicants.

From 2002, AFSSA contributed significantly to reinforcing the requirements that industrial applicants had to satisfy (in terms of data and tests), by identifying the sensitive aspects of health risk assessments related to the consumption of GMOs (AFSSA 2002). The Agency was the first in Europe to consider adapting the protocol for 90-day subchronic oral toxicity studies to the assessment of GMPs.

In addition, in 2011 ANSES issued an Opinion on methods for the statistical analysis of data for this study, which resulted in recommendations for the implementation of the protocols and analytical methods to be used to guarantee the reliability of results. In particular, this Opinion recommended increasing the number of animals to increase the statistical power of the tests (ANSES 2011).

3.1.2: Collective expert assessment process and requirements concerning the authorisation of plant protection substances

3.1.2.1: Process of collective expert assessment and authorisation of plant protection substances and preparations

The assessment of active substances in plant protection formulations, and of the formulations themselves with regard to marketing, is strictly regulated and harmonised at European level by Regulation EC 1107/2009[9], replacing Directive 91/414/EEC[10], which was in force until June 2011.

The process requires two phases:

- the **first phase**, carried out jointly by EU Member States, involves identifying the hazards of **active substances** and assessing the risks related to a reference product, with a view to ruling on whether or not these substances should be approved in Europe.
- the **second phase**, for approved active substances, consists in assessing the agricultural benefits and the risks related to commercial **formulations**; for this purpose, Europe is divided up into three geographical zones (North, Centre and South): France is in the South zone.

In France, applicants submit an MA[11] application dossier to ANSES's Regulated Products Department.
To investigate the application, ANSES:

- examines the data supplied and verifies their scientific validity as well as their compliance with regulatory requirements,
- assesses the agricultural risks and benefits related to the use of the formulation.

The investigation is carried out in accordance with the collective expert assessment principles applied by ANSES. When it is complete, the conclusions of the assessment, in some cases together with recommendations for management measures, are laid out in an Opinion. Conclusions relative to the acceptability of risk refer to the criteria indicated in Regulation (EU) 546/2011[12]. They are expressed as either "acceptable" or "unacceptable", with reference to these criteria.
The Directorate General for Food then uses the ANSES Opinion to decide whether or not to grant an MA or any modification of a current MA. This MA is issued when, under normal conditions of use associated with good agricultural practice, the formulation is deemed effective and free of unacceptable affects on human or animal health or the environment. The decision concerning MA details:

- the crop(s) targeted by this treatment,
- the pest(s), disease(s) or weed(s) targeted,
- the dose, period and frequency of application for the formulation, with any other agricultural practices associated with the treatment,
- restrictions concerning the conditions of use and management measures.

It should be noted that active substances and their associated formulations must be reassessed systematically according to a schedule laid down in Regulation (EC) 1107/2009.

[9] Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.
[10] Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market, transposed into French Law by the Order of 6 September 1994 implementing Decree 94/359 of 5 May 1994 on the control of plant protection products.
[11] MA: Marketing Authorisation
[12] Commission Regulation (EU) No 546/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards uniform principles for evaluation and authorisation of plant protection products.

3.1.2.2 Guidelines for the assessment of plant protection products

The implementing regulations for Regulation (EC) 1107/2009 (i.e. Regulation (EU) 544/2011[13] for active substances and Regulation (EU) 545/2011[14] for formulations) specify the information to be included in application dossiers and the methods to be followed to obtain it. These Regulations refer explicitly to methodology guidelines adopted by European or international organisations such as OECD, FAO[15] and EPPO[16] and are supplemented by almost 200 technical guidance documents, adopted at European level, detailing the models to be used, the parameters to be taken into account and the default values to be included in the models in the absence of valid information in the application dossier. These guidance documents are available from the European Commission website. The absence of required data or the presentation of non-compliant information result in a conclusion of unacceptable risk or in default values being used (if available), which is always disadvantageous for the applicant.

Regarding more specifically the data used for the assessment of risk to human health, the requirements are summarised below.

Applications concerning **active substances** must enable the intrinsic properties of these substances to be characterised and therefore the hazards they pose for humans and the environment. To assess the effects on human health, they must include full reports of the following toxicity and metabolism studies in mammals, carried out according to the guidelines defined by the regulations and good laboratory practice:

- metabolism studies in animals,
- acute toxicity studies for exposure by the oral or dermal routes or by inhalation,
- dermal or ocular irritation studies,
- skin sensitisation study,
- studies of toxicity by repeated oral administration in the short, medium and long term, and carcinogenic studies,
- mutagenicity studies,
- toxicity study for reproduction over two generations and studies on the effects on development,
- neurotoxicity studies depending on the properties of the substances,
- other studies depending on the results obtained from the preceding studies, especially for better identification of the effects and mechanisms of action.

The OECD guidelines for long-term toxicity studies and carcinogenic studies are summarised in Annex 2.
More specifically, to characterise the long-term effects of the active substance, such as those studied in the article by Séralini *et al.* (2012), the guidelines insist on two long-term and carcinogenesis studies performed on different species (rats and mice: 50 animals per group) to assess the general effects and potential carcinogenic effects of the substance when administered daily over the rodent's entire lifetime.

In these studies, numerous physiological, biochemical and histological parameters are monitored and measured in the animals. They enable a study of the dose-effect relationship, the toxicological mechanism of action, the reversibility of effects, whether or not there is a threshold for the undesirable effects, species specificity and the potential for extrapolating effects to humans.
On the basis of the most sensitive effects observed in these studies, toxicity reference values (ADI[17], ARfD[18], AOEL[19]) are calculated at the end of the European collective assessment for each

[13] Commission Regulation (EU) No 544/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the data requirements for active substances
[14] Commission Regulation (EU) No 545/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the data requirements for plant protection products
[15] FAO: United Nations Food and Agriculture Organization.
[16] EPPO: European and Mediterranean Plant Protection Organization
[17] ADI: the Acceptable Daily Intake of a chemical is an estimate of the quantity of the active substance in food or drinking water that can be ingested every day over the entire lifetime, with no appreciable risk to the health of the consumer, bearing in mind all known factors at the time of the assessment. It is expressed in milligrams of chemical substance per kilogram of body weight (WHO, 1997).

active substance. They will be used later to determine the risks associated with the use of plant protection formulations containing the substance.

Application dossiers must also contain study reports on metabolism and residues in the plants (and in foodstuffs of animal origin where concerned). For each foodstuff intended for human consumption, whether of plant or animal origin, the nature of the residue is defined (active substances and any relevant metabolite(s)). An MRL[20] is then determined for each active substance and each foodstuff, in order to ensure that consumer exposure remains below the values considered to be without risk to health in the short and long term.

A **plant protection formulation** consists of one or more active substances, most often associated with one or more co-formulants, which play a role in preparing or stabilising the formulation (water-based, powder, granules, suspension, etc.) or in modifying the availability of the active substance to the target pest (parasites or weeds). The MA application is submitted for one or more specific uses, a use being defined for the crop treated, the target pest, the quantity of product used per hectare, the period and frequency of use. Regarding human health, formulations are assessed for the risk to workers applying the treatment, agricultural workers handling the treated plant and bystanders[21], as well as the risks to consumers (chronic and acute risks to adults, toddlers[22] and infants, for different diets and for drinking water).
Marketing authorisation application dossiers must contain information enabling the characterisation of the formulations, of the concentration of active substances and co-formulants they contain and their associated hazards and in particular they must include full reports of the following toxicity and metabolism studies in mammals, carried out according to the guidelines defined by the regulations and good laboratory practice:

- Acute toxicity studies for the formulation, particularly to determine the toxicity of the product relative to the active substance and, if possible, the toxic mode of action, by the oral route, the dermal route and, if exposure by this route is possible, by inhalation,
- Dermal and ocular irritation studies,
- Skin sensitisation study.

The application dossier also contains toxicology data relative to the non-active substances.
Thanks to a process harmonised at European level[23], classifications based on a hazard assessment coordinated by the European Chemicals Agency have been published for a large number of substances and co-formulants.
The range of information available makes it possible to characterise the formulation and, in particular, to propose, on the basis of its composition in active substance(s) and co-formulant(s) and their properties, a classification corresponding to the hazards presented by this formulation.

The risk assessment takes into account the hazard determined for the formulation and the level of exposure, measured during tests or calculated using models.
The following tests must be carried out to estimate exposure for each formulation, and their full reports must be included with each application dossier:

- Dermal absorption study
- Tests for residues in the products treated and the derived food and feed.

In the particular case of plant protection formulations for treating GM crops that are tolerant to the active substance, residual tests on the GM crop concerned are required (Annex 3).

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[18] ARfD: The Acute Reference Dose of a chemical is the estimated quantity of a substance found in food or drinking water, expressed as a proportion of body weight, that can be ingested over a short period, usually in the course of a meal or a day, with no appreciable risk to the health of the consumer, bearing in mind all known factors at the time of the assessment. It is expressed in milligrams of chemical substance per kilogram of body weight (WHO, 1997).

[19] AOEL: The Acceptable Operator Exposure Level is the maximum quantity of active substance to which an operator can be exposed on a daily basis, with no hazardous effect on his/her health.

[20] MRL: The Maximum Residue Level.

[21] Persons who are located within or directly adjacent to the area where the pesticide application is in process

[22] Children from 13 to 18 months.

[23] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures.

At present, in accordance with the opinion of the European experts, the regulations do not require any long-term toxicity study on the formulated preparation (active substance + co-formulants). Such a requirement would necessitate a far greater number of tests on vertebrates, whereas it is currently considered that these should be limited to what is strictly necessary.
The long-term effects of the active substance are characterised and, as regards co-formulants, toxicological data are available. Regulation (EC) 1107/2009 includes a list (in its Annex III) of co-formulants that may not be included in the composition of plant protection formulations. This list is currently being compiled and will be updated to take into account any new knowledge about hazards. Any new information revealing a hazard is nonetheless already taken into account. For example, the use of polyethoxylated derivatives of nonylphenol, as a co-formulant in plant protection formulations and biocides, is prohibited[24], because of their endocrine-disrupting properties. In the case of insufficient data or of doubt about the long-term toxicity of a co-formulant, the provisions of the REACh[25] Regulation for the assessment of chemical substances authorise assessment agencies to ask applicants to carry out further studies.
Furthermore, the results of the acute toxicity studies conducted with the formulation are compared with the results expected in view of the known properties of the active substance(s) in order to identify any deviations raising questions about the toxicity of the co-formulants.

The issue of accumulated risk is the subject of much discussion at European level. ANSES has chosen an assessment methodology that it employs for plant protection formulations whenever several constituent substances have been classified as having properties that are carcinogenic, mutagenic or toxic to reproduction. This methodology, based on an approach involving target organs and mechanisms of action, is described in a memorandum available on the ANSES website.

Certain co-formulants can influence the exposure of humans to active substance(s) in the formulation, either during application, by acting on the way these substances are absorbed, or, for those consuming the foodstuff, by modifying the level of residues found in the treated crop. This is why applicants must systematically provide a dermal absorption study, together with tests designed to measure residue levels in the treated plants after treatment, conducted with the plant protection formulation.

3.2 An inventory of toxicology studies available on GMOs and glyphosate-based formulations

The toxicology studies needed for examining regulatory dossiers and applications for the authorisation of GMOs and plant presentation products, described in the previous section, are examined by the bodies responsible for ruling on these dossiers. The studies available during the regulatory investigation of NK603 maize and ROUNDUP are described in greater detail in the annexes concerning the assessment history of these dossiers (Annexes 3 and 4).
These regulatory studies are not always published in the scientific literature. On the other hand, other research teams use experimental protocols that are not directly linked to the initial authorisation of these products. These studies can be found in peer-reviewed scientific journals. An inventory of scientific publications useful in clarifying the questions raised by the study by Séralini *et al.* (2012) has been drawn up.

3.2.1 A literature search of relevant studies in the context of this Request and concerning genetically-modified plants

Several summary reviews have been compiled from the literature on assessment of the risks related to genetically-modified plants. A recent review was published in the journal "*Environment International"* in February 2011 by J.L. Domingo and Bordonaba (Domingo and Giné Bordonaba 2011). This summary describes the wide range of protocols used that are not systematically applied according to the recommendations in the international scientific literature (Domingo 2007). The

[24] Directive 2003/53/EC of the European Parliament and of the Council of 18 June 2003 amending for the 26th time Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (nonylphenol, nonylphenol ethoxylate and cement).

[25] Regulation (EC) No.1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals.

listed assessment protocols may have widely differing durations (from 28 days to 104 weeks), use various animal models (rats, mice, chickens, macaques, etc.) and investigate very different parameters (growth, pathological effects, biochemical parameters, etc.). This summary concludes that the number of publications specifically devoted to the assessment of the risks related to GMOs remains limited. However, it emphasises the fact that for the first time, a balance seems to be emerging between the number of authors who state that the genetically-modified plants studied are as safe and have the same nutritional properties as their respective conventional non-GM plants, and the number of authors raising concerns. This summary does not take a critical position regarding the validity of the interpretations of the authors cited, though certain among these have been questioned by the scientific world. It states, furthermore, that the studies concluding that GMPs are safe have often been carried out by companies responsible for marketing these GM plants.

A considerable number of studies described in this publication are tests referred to as 90-day subchronic toxicity studies that administer the product through feed, using part of the genetically-modified plant. The purpose of these tests is to assess the toxic potential of GMPs and the safety of subchronic consumption. These studies are based on the OECD's protocol 408, which is used to test the subchronic toxicity of chemicals in rodents. There are about 20 such publications (Annex 5) in the scientific literature. Most of these studies are carried out by major companies or by Contract Research Organisations (CROs) at the request of major companies and are frequently found in MA application dossiers, such as the one by Hammond *et al.* (Hammond *et al.* 2004) for the application concerning NK603 maize.
Three quarters of the GMPs tested are first-generation GMPs, i.e. resistant to insects and/or tolerant to herbicides. The animal groups contain between 10 and 20 subjects, three quarters of these being Sprague Dawley rats. The percentage of inclusion in the animals' diets varies according to the plant species tested. Maximum percentages are often 30% for maize and may reach 60% for rice. According to the authors, none of these studies has revealed any harmful effects on health.

Another review of the literature, published in 2011 in the journal "*Food and Chemical Toxicology*" (Snell *et al.* 2012) made a particular assessment of "the health impact of GMP diets in long-term and multigenerational animal feeding trials". It examined 12 long-term and 12 multigenerational studies. The description of these long-term studies confirms the variation in the protocols, species and results observed by (Domingo and Giné Bordonaba 2011) for all the studies. They involve a wide range of species (rats, mice, dairy cows, salmon, macaques) and the observation period varies from 26 to 104 weeks for species with varying lifetimes. The vast majority of these studies concerned glyphosate-tolerant soybeans, and none were performed on NK603 maize.
The authors of this summary conclude that:

- these 24 studies suggest that there is no particular hazard and that, although differences are sometimes observed between the control animals and the animals fed with the GMPs studied, these can be explained by the range of biological variation naturally observed between individuals of the species. According to the authors, they do not show any evidence of a toxic effect of GMOs,
- none of these studies describe observations that would require further information to be provided in addition to the 90-day subchronic toxicity studies on rodents laid down by OECD guideline 408 (OECD 1998). The 90-day subchronic toxicity studies would seem to be sufficient as a basis for regulatory assessment of GMOs. However, one cannot rule out the possibility that long-term studies should be carried out on a case-by-case basis for regulatory assessments if reasonable doubts persist after an examination of the 90-day subchronic toxicity study.

Protocol for a literature search of relevant studies in the context of this Request and concerning NK603 maize
In view of the questions asked, the literature search focused on identifying all studies concerning NK603 maize. It was broadened to include all genetically-modified plants carrying the CP4*epsps* gene and long-term studies concerning all genetically-modified plants.
The investigation particularly sought any new publications not referenced in previous reviews and likely to provide input to this investigation. Two databases were searched (Scopus© and Medline) with the use of keywords.

The results were compared with the references cited in the summary literature reviews (Domingo and Giné Bordonaba 2011; Snell *et al.* 2012) and ANSES's own literature on the subject. No new publications were identified that had not been previously considered, with the exception of the article by Séralini *et al.* that is the subject of this Request.

Only two studies, carried out over a period close to the average lifetime of the animals concerned, using a glyphosate-resistant GMP (soybeans) and which could therefore be compared to the study by Séralini *et al.* (2012), were identified. These are studies by Sakamoto et al. (Sakamoto *et al.* 2008) and Malatesta et al. (Malatesta *et al.* 2008). ANSES commissioned an English translation of the paper by Sakamoto *et al.* which had been published in Japanese.

3.2.2 A literature search of relevant studies in the context of this Request for glyphosate-based formulations

Several studies were examined during the investigation of the active substance glyphosate in view of its inclusion in Annex I of Directive 91/414/EEC, and the MA application dossiers for the plant protection formulations containing this substance. Annex 3 summarises the data taken into account, some of which, found in the literature, were produced by the American National Toxicology Program (NTP).
In view of the questions asked, the literature search focused on identifying all studies concerning glyphosate-based formulations. It was also broadened to include glyphosate, co-formulants associated with it in ROUNDUP formulations, and long-term studies. The investigation particularly sought any new publications not referenced in previous review and likely to provide input to this investigation. Two databases were searched (Scopus© and Medline) with the use of keywords (ROUNDUP, glyphosate, long term studies, 104-week study, toxicity).

Regarding more specifically the long-term studies (lasting for one year or more), there are no experiments reported in the scientific literature carried out on ROUNDUP GT PLUS, nor on other glyphosate-based formulations, nor with the co-formulant found in the formulation ROUNDUP GT PLUS.

3.3 Critical analysis of the most relevant long-term studies

3.3.1 Description of the studies

3.3.1.1 The study by Malatesta *et al*.

3.3.1.1.1 Objective and study protocol

The study by Malatesta *et al.* (Malatesta *et al.* 2008) examines the effects on the liver of a diet containing 14% genetically-modified soybeans (CP4 EPSPS event) treated with ROUNDUP, administered for two years to female Swiss mice (10 mice per group). It is not a regulatory toxicity study but a study undertaken for research purposes that therefore does not fall within the scope of the OECD guidelines. This study combines a proteomic approach to determine whether a GM diet leads to protein level changes in the liver with the investigation of morphological and cellular parameters. In this framework, the number of mice (ten female mice per group) appears comparable to that used in other publications having the same objective.

3.3.1.1.2 Results

According to the authors, the GM diet did not result in any significant differences in mortality or animal and liver weights. Moreover, no macroscopic alterations, pathologic lesions or onset of tumours were observed in the mice's organs.
As far as proteins were concerned, no differences were observed in the total protein content of the liver and the number of identified proteins, which remained stable (approximately 1400). However, the proteomic analysis found 49 proteins that were expressed differently in the GM-fed mice, including 39 that were up-expressed and 10 down-expressed. Twenty of these proteins were identified by mass spectrometry and were proteins involved in hepatocyte metabolism, stress response, calcium pathways and mitochondrial function. At the morphological level, the authors

report nuclear and mitochondrial changes that appeared only after the first year. The authors conclude that GM soybeans affect the metabolic capacity of hepatocytes and the ageing process. They underline the importance of undertaking research into the long-term effects of a GM diet.

3.3.1.2 The study by Sakamoto *et al*.

3.3.1.2.1 Objective and study protocol

Sakamoto *et al.* (Sakamoto *et al.* 2008) assessed the long-term safety of genetically-modified soybeans (CP4 EPSPS event) in F344 rats fed a diet containing these soybeans at a concentration of 30% for two years. Three groups of rats were used. They were respectively fed a diet containing 30% genetically modified soybeans (50 rats/sex), a diet containing 30% near-isogenic soybeans (50 rats/sex) and a standard soybean-free diet (35 rats/sex). Of the long-term toxicity studies found in the literature, the one by Sakamoto *et al.* is the closest to a regulatory study. Most of the recommendations in OECD protocol 453 were followed, particularly in terms of the large number of animals (50/group).

3.3.1.2.2 Results

At the end of the study, the authors did not note any differences in survival rates, body weights or food intake related to GM soybeans. Some haematological parameters (haemoglobins, haematocrit levels, mean corpuscular haemoglobin concentration) were significantly decreased in the GM group compared to the non-GM group, but these variations were all below 4% and not biologically relevant. The only biochemical (transaminases, creatinine) and organ weight changes involved soybean treatments *versus* a standard diet. The only significant differences in the onset of neoplastic tumours were also observed in animals fed a soybean diet *versus* a standard diet. In conclusion, this study shows that GM soybeans administered to F344 rats for two years did not lead to significant changes compared to rats fed non-GM soybeans. However, a diet made of 30% soybeans may have potentially harmful effects.

3.3.1.3 The study by Séralini *et al.*

3.3.1.3.1 Objective and study protocol

The study by Séralini *et al.* (2012) examined for rats the long-term dietary toxicity of ROUNDUP GT PLUS and a glyphosate-tolerant maize administered via their feed. The glyphosate-tolerant maize used in the study contained the NK603 transformation event.
Over a two-year period, ten groups of ten rats of each sex were fed a diet containing either:

- 33% non-GM maize (control group),
- 11, 22 or 33% NK603 maize not treated with ROUNDUP WEATHER MAX,
- 11, 22 or 33% NK603 maize treated with ROUNDUP WEATHER MAX,
- 33% control maize and drinking water containing three different doses of ROUNDUP GT PLUS.

The authors monitored rat mortality and tumour incidence in each group. They performed histological staining and electron microscopy on organs presenting a pathology during the experiment. The publication presents a discriminant statistical analysis (OPLS-DA[26]) of biochemical blood and urine parameters (measured at 15 months) in the group of females fed 22% NK603 maize not treated with ROUNDUP WEATHER MAX compared to the control group.

3.3.1.3.2 Results

The authors describe earlier and higher mortality and tumour incidence in all of the treated groups compared to the control group for females and in the GM-fed groups for males. The observed pathologies were sex-dependent mainly with mammary tumours and pituitary abnormalities in

[26] Orthogonal Partial Least Squares-Discriminant Analysis

females and pathologies related to the liver, hepatodigestive tract and kidneys in males. Images of histopathological and electron microscopy sections illustrate these results. Ferulic and caffeic acid levels in the rats' diets were lower in the chow (pellet feed) containing NK603 maize. Biochemical data for the female group fed 22% untreated NK603 maize and the control group were analysed using a statistical method that shows that the most discriminant variables for the two groups are kidney related. Blood oestrogen levels were modified. According to the authors, the study's results are due to hormone-dependent food toxicity, non-linear in relation to the dose, having different effects for each sex.

These three study protocols are summarised in the following table:

	Séralini *et al.*	Sakamoto *et al.*	Malatesta *et al.*
PROTOCOL			
Species and Strain	Sprague Dawley rats	F344 rats	Swiss mice
Study period	2 years	2 years	2 years
Number of animals/group/sex	10	4 x 50 and 2 x 35	10
Number of groups	20	6	2
Total number of animals	200	270	20
Sex	Male and female	Male and female	Female
Plant material	NK603 maize	ROUNDUP Ready soybeans	40-3-2 soybeans
Transgene	two CP4-epsps genes	one CP4-epsps gene	one CP4-epsps gene
Protein	two CP4-EPSPS proteins	CP4-EPSPS	CP4-EPSPS
ROUNDUP plant treatment	Depending on the group	Not specified but presence of glyphosate residues	Yes
Tested doses for the various groups (**abbreviations used in the text**)	11%, 22%, 33% NK 603 maize seeds not treated with ROUNDUP **(% GMO**) 11%, 22%, 33% NK 603 maize seeds treated with ROUNDUP WEATHER MAX (3 L/ha) **(% GMO + R**) 50ng/L **(RA)**, 400mg/kg **(RB)**, 2.25 g/L **(RC)** of ROUNDUP GT PLUS in drinking water	30% soybeans	14% ROUNDUP-treated soybeans
Doses in the control groups	33% near-isogenic	30% near-isogenic Soybean-free diets	14% commercial soybeans
Feed composition analysis/balance between the groups	Yes but not shown Hearing: Absence of mycotoxins (< LOQ)	Yes	No
Age at start of experiment	5 weeks	4 weeks	Age at weaning
COLLECTED DATA			
Presence of the transgene in the plant	Yes	Yes	No
Consumption data	Not provided	Yes	No
Growth data	Not provided	Yes	No
Mortality	Yes	Yes	No
Organ weight	No	Yes	The liver only

	Séralini *et al.*	Sakamoto *et al.*	Malatesta *et al.*
Anatomical pathology tests	Yes Unclear number and description of histopathological observations	Yes Clear number and description of histopathological observations (non-neoplastic lesions, number of hepatocellular foci, nephropathies, neoplastic lesions) Histopathologies	No
Other tests	Electron microscopy		Liver examinations: Proteomic analysis Electron microscopy Cell morphometry Immunohistochemistry
Tumour frequency	Yes	Yes	No observed tumours
Onset time	Yes	No	
Biochemical parameters	Yes (haematology, blood and urine biochemistry)	Yes (haematology, blood biochemistry)	No
Organs weight	Not provided	Yes (8 in males and 9 in females)	Yes (liver)
Steroid hormones	Yes in the blood	No	No
Behavioural analysis/clinical follow-up	Twice a week: observation and palpation, recording of clinical signs	Daily observation	No
Ophthalmological tests	Yes	No	No
Statistical data processing	No statistical tests on treatment differences for mortality and pathology incidence. Discriminant analysis based on the OPLS method for biochemical data. No estimation of dose/sex/ROUNDUP GMO effects or calculation of confidence intervals for these effects	Statistical analysis consistent with the OECD recommendations (mean and frequency comparisons, calculation of confidence intervals) For growth, intake, organ weight, biochemical and haematological data Student's t-test for comparing GM and non-GM groups	GMO/Control difference tests for proteomics

3.3.2 Critical analysis of the literature

3.3.2.1 The study by Malatesta *et al*.

This an original study that assesses the effects of GMOs on nonstandard parameters using sophisticated techniques (proteomics and electron microscopy). In this study, the soybeans administered to the control group are poorly defined and are not near-isogenic non-GM soybeans. Moreover, none of the groups received non-herbicide-treated GM soybeans, which could have distinguished effects related to the latter. The analysis focused solely on the detection of liver changes. This study shows the effects of administration for two years of a diet containing GM soybeans on the morpho-functional characteristics of hepatocytes. However, the biochemical blood and urine parameters traditionally assessed to express the harmful effects on this organ for toxicological purposes are not presented.
In the end, in spite of the observed molecular and cellular differences, the Swiss mice that were exposed for 24 months to a diet containing 14% GM soybeans treated with ROUNDUP did not

show signs indicating any carcinogenesis process. That said, this study was undertaken in a line of mice (Swiss) that is known in the literature for developing very few tumours (Annex 6).

3.3.2.2 The study by Sakamoto *et al*.

The study is the closest to a standard carcinogenicity study protocol in rats and uses the recommended number of animals. It can be criticised for not measuring renal function parameters and a lack of information about tumour-onset times.
Regarding the study's experimental protocol, it is not specified whether or not the GM soybeans were treated with a glyphosate formulation. However, the trace amounts (0.1 ppm) of glyphosate detected in these soybeans suggest that this was the case (Sakamoto *et al.* 2007). If so, like in the above study, it is unfortunate that there are no GM groups not treated with a glyphosate formulation in addition to the other groups. In terms of statistical analysis, the methods used are consistent with the OECD's recommendations, with mean and frequency comparisons and a calculation of confidence intervals to compare the group fed the GM soybeans with the control groups (non-GM soybeans and soy-free diet). In the end, no biologically significant differences were observed between the various groups of rats that may reflect harmful effects related to the genetic modification of soybeans.

3.3.2.3 The study by Séralini *et al*.

Considering the subject matter of the Request, this study has been analysed in detail.

3.3.2.3.1 Protocol

A broad study
The study by Séralini *et al.* (2012) was undertaken in an experimental research framework and was not intended to be strictly compared to studies undertaken for the authorisation of products and substances (regulatory studies). Domingo *et al*. (Domingo 2007) confirm that a number of GMO publications are based on studies that do not follow the guidelines recommended in the context of these regulatory studies.

With regard to such research protocols, the study by Séralini *et al.* (2012) is an ambitious study that was undertaken with considerable resources. It is worth highlighting on account of its originality; indeed, very few publications describe work examining both the long-term effects of GMOs and the herbicide to which they are tolerant.
This study is unique in that over this long period and using several doses, it tests both a GMP cultivated with and without treatment by a plant protection product and the complete plant protection formulation by itself. In this respect, no equivalents have been found in the literature. It is also distinctive in that it monitors a large number of blood and urine parameters and the authors indicate that it was undertaken in a GLP environment[27].

The main criticisms that the authorities have made thus far involve a lack of information in the publication about the composition of the feed and the types of tested diets, the choice of doses, the strain of rat, the number of rats per group and the statistical analysis of data.

Missing information
During the hearing, Mr. Séralini's team answered a number of questions regarding data that do not formally appear in the publication. They provided the missing information or offered guarantees in relation to the following:

- The periods and number of crop treatments with ROUNDUP WEATHER MAX and other plant protection products for the maize grown in this study,
- The chemical composition of the seeds and their levels of mycotoxins, glyphosate and its residues,

[27] The claim for GLP status made for the study published by Séralini *et al*. (2012) implies application of the principles listed in the OECD ENV/JM/MONO (2002) document addressing the specific case of studies using multiple sites.

- The composition of the diets and the fact that the feed of the rats in the 11% and 22% groups was supplemented with near-isogenic maize to reach 33% maize in the feed,
- The feed storage method.

The choice of doses

Regarding the doses of GMOs in the feed, which were 11%, 22% and 33%[28], the study protocol is comprehensive and standard. These doses (11% and 33%) correspond to those generally used in regulatory 90-day subchronic toxicity studies in rats.

However, the levels of ROUNDUP GT PLUS administered in the drinking water of the rats raise two points. The first involves the scale of variation between the three tested doses, which range from 50 ng/L to 2.5 g/L, i.e. a multiplicative factor of around fifty million. The gap between the high doses and the low dose is therefore too large to be able to determine a dose-response relationship. The second point involves the relevance of these three doses in terms of exposure in consumers and users of glyphosate formulations.

The three concentrations tested in this study were compared to the available exposure data:

- The first tested dose corresponds to a level that the author describes as the glyphosate contamination level in tap water: 50 ng/L. The regulatory standard in France stipulates no more than 100 ng/L in drinking water. For the water supply, of 43,741 tests that screened for glyphosate (2007-2009 period)[29], only 95 (0.2%) detected quantifiable levels of glyphosate. These quantifiable results were found in a limited number of distribution stations (0.2 to 0.4% of the 21,864 tested stations). 50 ng/L is therefore a realistic value that could potentially be observed but only in a very limited number of French stations. Moreover, these analytical data apply only to glyphosate and not glyphosate combined with co-formulants. The ROUNDUP GT PLUS co-formulant is not mobile in the soil (Koc = 2500 to 9600, DT50 soil = 1-2 days)[30]. The likelihood of finding the tested quantities in groundwater appears negligible.

- The second tested dose corresponds to a contamination level that the author describes as ‘equivalent’ to the US MRL for glyphosate in GM feed (400 mg/kg). In Europe (source: the DG Sanco website), the MRL for glyphosate is set at 0.1 mg/kg for sweetcorn (as a vegetable-fruit) and 1 mg/kg for maize as a cereal. Therefore, the level to which European consumers are exposed is far lower than the level tested in this protocol. Furthermore, as in drinking water, since co-formulants are not systemic, consumers will primarily be exposed to glyphosate and not glyphosate combined with a co-formulant.

- The third tested dose corresponds to a level that the author describes as "half of the minimal agricultural working dilution" (2.25 g/L). Taking into account the concentration of glyphosate in the formulation (ROUNDUP GT PLUS), the quantity to be applied per hectare (data from the French Ministry of Agriculture's E-phy database) and the dilution recommended by the manufacturer, the level of glyphosate in spray mixtures would be approximately 7 g/L, which is the same order of magnitude as the dose tested in the publication. However, users will mainly be exposed to the diluted ROUNDUP GT PLUS formulation through dermal contact and potentially inhalation. The route of administration described in the study protocol (oral exposure) is therefore not the most appropriate for assessing the risks related to the product's application.

[28] Extrapolated to humans, the dose of 33% corresponds to a daily dose that is approximately 40 times higher than average intakes of maize. Monsanto indicates a proportion 84 times higher in its submitted document.

[29] Source: the French Ministry of Health's SISE-Eaux database

[30] JP Giesy, S Dobson and KR Solomon (2000) Ecotoxicological Risk Assessment for Roundup Herbicide, Rev. Environ. Contam. Toxicol., 167:35-120

Number of rats and rat strain
During their hearing, the authors of this study reiterated that one of the study's initial objectives had been to assess the ability of 90-day subchronic toxicity studies to predict the onset of long term effects.
In this framework, it seemed appropriate to choose Sprague Dawley rats, which are the most frequently used rats for this protocol[31] and the choice of ten rats per group was justified because it is commonly used in the context of subchronic toxicity studies.
It should be noted that the number of rats per group is a decisive factor when attempting to prove the safety of a product (regulatory studies) because it determines statistical test power and the probability of detecting an effect. By using a small number of animals per group, the authors ran the risk of not being able to find statistically significant differences between the groups and therefore of having conducted an inconclusive study, considering the study's duration, which was 2 years[32] instead of 90 days, and the susceptibility of the rat strain used.

The data in the literature on Sprague Dawley rats (Annex 6) show high mortality rates and high incidence rates for mammary tumours in control groups, which were the main abnormalities observed by Séralini *et al.* (2012). These phenotypic characteristics should have been taken into account when calculating the required number of animals.

Size and number of control groups
A significant criticism is the use of only one control group and the small number of male and female control animals which considerably limit interpretations of this study. During the hearing, the study's author agreed that this point was unfortunate.

3.3.2.3.2 Results and discussion

For information, the authors describe more and earlier deaths in the female population in all treated groups and 'generally'[33] earlier and larger tumours. They describe liver damage for all of the GM-treated males.
The main criticism of the study's results concerns the lack of statistical data analysis supporting these findings.

The authors simply note that the treated groups were generally more affected than the control group without testing the possibility that these results may have been due to chance. The authors, when asked about this point, indicated that they had simply wanted to report their results, which they had found disturbing, not in the form of a statistical analysis but rather in the form of a description as practised in human clinical research.

> *"Gilles-Éric Séralini's team added that this study corresponded to a research protocol and was not at all intended to be a regulatory test protocol. The results are presented factually for both tumours and mortality. The team did not wish to conduct statistical analyses for these points, as it was keenly aware that with 10 rats per group, tests would not be sufficiently powerful. The team had criticised the Monsanto study for just this. Gilles-Eric Séralini stated that thorough statistical analyses had however been undertaken for biochemical parameters, confirming disturbances that can lead to the observed pathologies" (Extracted from the verbatim report of the hearing with the study's authors)*

Following the hearing, ANSES asked Mr Séralini to submit all of the study's raw data. The authors did not grant this request (see hearing) but submitted quantitative data on mortality and the onset of non-regressive tumours. The ECEAG was able to use some of these data (on mortality) in addition

[31] OECD protocols 452 and 453 are not prescriptive with regard to the rat strain to be used and while the Fischer 354 strain is often used in carcinogenicity studies, mainly because it is the best known, the Sprague Dawley strain is currently being evaluated by the National Toxicology Program (NTP).
[32] As two years is a rat's average lifespan, a high percentage of animals risk dying before the end of long-term studies.
[33] almost always more often

to those available in the publication (Figure 1 and Table 2) to determine the significance of certain results.
In order to increase the probability of detecting effects, the ECEAG first undertook one-tailed tests[34] considering Type I error risks[35] of 5% without taking the effect of multiple testing into account. This approach increases statistical power and yet also increases the risk of false positives (false discovery). It is considered the most favourable statistical test for highlighting a maximum number of effects that need to be interpreted biologically. The statistical tests were then corrected so as to limit risks of false discoveries (FDR (False Discovery Rate) control or correction)[36] (Benjamini and Hochberg 1995). Indeed, undertaking multiple statistical tests on a given dataset rapidly increases the rate of Type I errors, i.e. the probability of observing falsely significant differences.

Three series of statistical tests were undertaken by the ECEAG:
The first series of statistical tests aimed to determine whether there were significant differences in mortality rates at the end of the study between the control group of rats and the GMO and/or ROUNDUP groups of rats[37]. These tests compared the null hypothesis H0 'Mortality rate in the control group = Mortality rate in the GMO and/or ROUNDUP groups' and the alternative hypothesis A 'Mortality rate in the control group < Mortality rate in the GMO and/or ROUNDUP groups'. This series of tests was undertaken using the data extracted from Figure 1 of the study by *Séralini et al. (2012).* The risk of a Type 1 error (probability of wrongly rejecting H0) was calculated by conducting Fisher's exact test on 2 dead rats out of the 10 rats of the female control group and 3 dead rats out of the 10 rats of the male control group. The results (Table 1) show that the differences in mortality rates are significant at the 5% level before correction (FDR) for two in 18 groups of rats:

- for the female group, GMO at the 22% dose,
- and for the female group, GMO + R at the 22% dose.

When taking multiple testing into account (FDR), no significant differences are found.

Table 1. Results of statistical tests (P=probability of a Type I error) on mortality rates. *The P values have not been corrected to take multiple testing into account. When these corrections are applied, no differences are significant.*

	Males		Females	
Group	Mortality rate	P-value	Mortality rate	P-value
Control	3/10	NA	2/10	NA
GMO 11%	5/10	0.3250	3/10	0.5
GMO 22%	1/10	0.9567	7/10	***0.0349***
GMO 33%	1/10	0.9567	4/10	0.3142
GMO 11%, R	4/10	0.5000	4/10	0.3142
GMO 22%, R	5/10	0.3250	7/10	***0.0349***
GMO 33%, R	3/10	0.686	4/10	0.3142
RA	3/10	0.686	5/10	0.1749
RB	4/10	0.5000	5/10	0.1749
RC	1/10	0.9567	4/10	0.3142

The second series of tests aimed to determine whether the rats in either of the GMO and/or ROUNDUP groups died earlier than the rats in the control group. The ECEAG used the Log-Rank test for this purpose. This test compared survival probabilities for the various groups. Three series of comparisons were conducted successively: Control *vs.* GMO alone, Control *vs.* GMO treated with ROUNDUP WEATHER MAX, Control *vs.* ROUNDUP GT PLUS. The tests were undertaken with

[34] Tests that assume that a difference will be in a particular direction. For this analysis, the assumption is that the groups treated with GMOs or ROUNDUP are likely to have adverse effects but not positive effects.
[35] Probability of wrongly rejecting hypothesis H0, which is the hypothesis that there are no differences between the groups.
[36] A procedure that controls the risk of false positives related to a high number of tests undertaken with the same data.
[37] Tested doses in the groups:
11%, 22%, 33% NK 603 maize seeds not treated with ROUNDUP **(% GMO**)
11%, 22%, 33% NK 603 maize seeds treated with ROUNDUP WEATHER MAX (3 L/ha) **(% GMO + R**)
50ng/L **(RA)**, 400mg/kg **(RB)**, 2.25 g/L **(RC)** of ROUNDUP GT PLUS in drinking water

and without correction (Sidak correction) for the number of comparisons made per series using the raw mortality data submitted by the author after his hearing. The results (Table 2) show that there are two significant differences out of 18 with the uncorrected tests:

- for the female group, GMO at the 22% dose,
- and for the female group, GMO + R at the 22% dose.

After correction, no differences are significant.

Table 2. Results of Log-Rank tests on reduced life expectancies with and without correction for multiple testing.

Comparison		Males		Females	
		Uncorrected	Corrected (Sidak)	Uncorrected	Corrected (Sidak)
GMO 11%	Control	0.661	0.9999	0.4522	0.9956
GMO 22%	Control	0.2357	0.911	**0.0159**	0.1341
GMO 33%	Control	0.0907	0.5751	0.122	0.6899
GMO 11%, R	Control	0.4797	0.9972	0.3233	0.9702
GMO 22%, R	Control	0.8953	1	**0.0448**	0.3378
GMO 33%, R	Control	0.7233	1	0.4666	0.9965
RA	Control	0.5778	0.9996	0.0841	0.5464
RB	Control	0.3179	0.968	0.239	0.9144
RC	Control	0.084	0	0.2501	0.925

The third series of tests aimed to determine whether the frequency of pathologies was higher in the GMO and/or ROUNDUP groups than in the control group. These tests compared the null hypothesis H0 ‘Frequency of pathologies in the control group = Frequency of pathologies in the GMO and/or ROUNDUP groups' with the alternative hypothesis A ‘Frequency of pathologies in the control group < Frequency of pathologies in the GMO and/or ROUNDUP groups’. These tests were undertaken using Table 2 of the study by Séralini *et al.* (2012) for the six listed pathologies with the six GMO treatments (three doses of GMO without ROUNDUP + three doses of GMO with ROUNDUP WEATHER MAX) and the three ROUNDUP GT PLUS treatments. The Type I error risk was calculated separately for each pathology and each diet with Fisher’s exact test. The results are shown in Table 3 below.

Of the 54 comparisons, five are significant at a level of 5% before FDR correction.

- ‘hepatic pathologies’ described by the author as liver congestions, macroscopic spots and microscopic necrotic foci
  - for the males in the group fed 22% GMO,
  - for the males in the RB group.
- mammary tumours
  - for the females in the RB group.
- pathological signs in the mammary glands (other than tumours described by the authors as galactoceles and mammary hyperplasias)
  - for the females in the RA group,
  - for the females in the RB group.

After FDR correction for multiple testing, there are no significant differences at the 5% level.

Table 3. Results of statistical tests (P = probability of a Type I error) on the incidence of pathologies (percentage of animals with at least one tumour or pathological lesion). The probabilities have not been corrected to take multiple testing into account. When these corrections are applied, no differences are significant at the 5% level.

Organs and associated pathologies	GMO 11%	GMO 22%	GMO 33%	GMO 11% + R	GMO 22% + R	GMO 33% + R	RA	RB	RC
Males, in liver	0.31	**0.035**	0.085	0.31	0.31	0.18	0.18	**0.035**	0.18
In hepatodigestive tract	0.5	0.33	0.5	0.5	0.5	0.33	0.07	0.18	0.67
Kidneys, CPN	0.5	0.33	0.09	0.33	0.5	0.5	0.19	0.33	0.67
Females, mammary tumours	0.33	0.33	0.18	0.5	0.33	0.07	0.07	**0.02**	0.07
In mammary glands	0.18	0.33	0.18	0.18	0.18	0.07	**0.02**	**0.02**	0.07
Pituitary	0.15	0.31	0.82	0.15	0.91	0.5	0.31	0.5	0.5

In general, more specific information about the observed pathologies is required to determine the biological significance of the statistical results before correcting for multiple testing as recommended, particularly in the ANSES report (ANSES 2011). The ECEAG considers it unfortunate that the definitions of the groups of pathologies described in the publication are unclear and that there are so few useable biochemical data. Nonetheless, the statistical analysis results as a whole show that:

- The increased mortality and reduced life expectancy (Tables 1 and 2) observed for the females in the GMO 22% and GMO 22% + R groups (before correction) are not confirmed by any underlying pathologies (Table 3). This finding is striking and additional information on the cause of death for each animal in these groups would be necessary to interpret it.
- The increase in pathologies highlighted in the publication is significant at a level of 5% (before correction) for only a small number of treatments and is difficult to interpret from a biological standpoint due to the unclear definitions of the pathologies.
- The increase in the incidence of hepatic pathologies in the ‘GMO 22% male' group (before correction) is not found at the 11% and 33% doses nor for the ‘GMO 22% + R male’ group. This result does not appear coherent since it occurs at a single intermediate dose and is not found in a group fed the same percentage of GM maize.
- The increase in hepatic pathologies observed in males for the RB dose of ROUNDUP GT PLUS (before correction) may be consistent with the LOAEL for glyphosate. However, the other pathologies (mammary tumours, galactoceles and mammary hyperplasia) observed in females at the RB dose do not appear consistent with the toxicological data on glyphosate (long-term studies in rodents). Furthermore, none of these effects are found at the highest dose (RC). This finding does not support biological coherence even though it could be expected that this high dose would interfere with the eating behaviour of rats. It would be useful to have data on the water and feed consumption of the treated animals.
- The significant increase in the frequency of mammary gland pathologies (excluding tumours) (Table 3) observed at the lowest dose of ROUNDUP GT PLUS (RA) (before correction) caught the attention of the ECEAG and may suggest an unexpected effect at a very low dose. However, in order to determine a biologically significant effect, it is necessary to have individual data, comprehensive biochemical data and historical data on the SD strain provided by the CRO.

Conclusions on study results

The significant results obtained before correction are not biologically coherent overall. However, biological data on the results would be needed to draw a definitive conclusion. At this point in time, in light of the information provided in the publication, the ECEAG's experts consider that the authors' interpretations are not sufficiently corroborated by the study data.

Moreover, during the hearing, the study's authors admitted that this study was not conclusive by itself and that, though subject to improvement, it had the merit of opening up an interesting line of research.

> *"The team's members firmly believe that, having used all techniques available, what they observed was not random. The study could certainly be improved but the team simply opened up a path and we must now collectively do better. "For Gilles-Éric Séralini's team, there is endocrine disruption because there is disruption of testosterone/oestradiol ratios and female pituitary glands in particular. These experiments need to be repeated since this was the first time that tests were undertaken with a pesticide as a whole at a low dose*" (*Extracted from the verbatim report of the hearing with the study's authors)".*

3.3.2.3.3 Assumptions

The mechanistic assumptions put forth by the authors are not corroborated by results and are therefore speculative. The ECEAG's members nonetheless considered it would be worthwhile to further discuss the merits of these assumptions.

Plausibility of an endocrine disrupting effect and low-dose effects

The assumption put forth by Séralini *et al.* (2012) to explain the development of mammary tumours in females is a mechanism of action related to endocrine disruption. According to the authors, this assumption is based on:

- variations in circulating levels of oestradiol and testosterone in the females in the treated groups,
- the onset of tumours in hormone-sensitive tissues (mammary and pituitary glands) in the treated groups,
- the results of prior studies published by the same team reporting *in vitro* effects on aromatase (an enzyme that converts testosterone to oestradiol) activity with ROUNDUP and those published by other authors (Romano *et al.* 2012; Romano *et al.* 2010; Walsh *et al.* 2000) reporting the effects of ROUNDUP on steroidogenesis, reproduction and development,
- relatively low levels of caffeic and ferulic acids in foods made with genetically-modified maize, which could lead to endocrine disruption,
- non-monotonic dose-response curves considered a characteristic of endocrine disruption

A close examination of the publication indicates that this assumption is not sufficiently corroborated by the study results. Indeed, judging by Figure 5 and Table 3, which show circulating levels in female rats at 15 months, the reported values do not indicate any significant effects for the treatments and there is no link between the observations made for the pituitary and mammary glands. Moreover, it should be noted that hormone levels in female rats vary considerably over the oestrous cycle and depending on the time of sampling during the day, which makes it difficult to interpret the data without having precise experimental details on the sampling conditions. Other hormones (*e.g.* prolactin, LH, FSH), hormone-sensitive tissues (testicles, ovaries, adrenal glands) and enzymatic activities involved in steroidogenesis would need to have been examined to draw any conclusions. Although the assumption of endocrine disruption with ROUNDUP has already been described in the literature (Romano *et al.* 2012; Romano *et al.* 2010), this article offers no evidence of these effects. Furthermore, on the basis of current knowledge, it is difficult to agree with the arguments put forth by Séralini *et al.* regarding an endocrine disrupting mechanism to explain (unproven) effects on mammary tumours related to the consumption of NK603 maize without exposure to a glyphosate formulation. Although most endocrine disruptors have effects that do not correspond to a monotonic curve, the lack of a dose-response relationship for the 'GMO' and 'GMO + R' groups can in no case be regarded as evidence of endocrine disruption.

Plausibility of effects related to secondary metabolites

In addition to its 'own' EPSP synthase, NK 603 maize contains a bacterial EPSP synthase (encoded by two copies of the CP4 EPSPS gene from *Agrobacterium tumefaciens*). This bacterial enzyme is glyphosate-tolerant and involved in a very early stage of the so-called 'shikimic acid' pathway (Annex 8). The authors suggest disruptions to the secondary metabolism of plants caused by genetic modification. Changes in the chemical composition of GMPs do indeed have to be documented as part of authorisation applications. In this context, each application must include a comparative analysis of the chemical composition of the GMP and that of its non-GM control.

Thus, differences in levels of certain secondary metabolites, and particularly phenolic acid metabolites, are noted and highlighted by the authors. These metabolites can have protective or endocrine disrupting effects.

The data presented in the article involve two types of compounds measured in rat diets (chow - pellet feed): isoflavone phyto-oestrogens and two phenylpropanoids: caffeic acid and ferulic acid. The authors indicated during the hearing that they had other data for other compounds (e.g. tocopherols) that had not yet been made available to the scientific community.

Regarding isoflavones: the authors did not observe any differences between the diets used in the experiments for these compounds. Isoflavone levels in maize are extremely low (< or << 100 µg/kg) (Kuhnle *et al.* 2009). Isoflavones are compounds that are known for being *selective oestrogen receptor modulators* (which largely explains their so-called 'phyto-oestrogen' properties). They are characteristic of food plants such as soybeans, yet their levels in maize are not in principle high enough to explain any protective or endocrine disrupting effects (AFSSA/AFSSAPS 2005)[38].

The phenylpropanoids measured in the study are caffeic acid and its *O-methyl* counterpart, ferulic acid. The latter has higher levels in maize by far (~ 90% of the total phenol content, ~ 50 times more than caffeic acid). Ferulic acid is a secondary metabolite that has traditionally been measured to compare the chemical composition of GM maize and its controls, together with other compounds (phytic acid, trypsin inhibitors, vitamin E, coumaric acid and raffinose). Maize can be described as a plant that is rich in ferulic acid (total ferulic acid content of around 1-3 g ferulic acid/kg of maize, dry-weight) (Ridley *et al.* 2002); (De La Parra *et al.* 2007) (OECD 2002). The levels reported by the authors (and measured in the diets containing 33% maize), once extrapolated to the levels in the initial maize, are consistent with the data in the literature.

In the specific case of NK603 maize, Ridley *et al*. (Ridley *et al.* 2002) analysed the two types of maize (NK603 *vs* non-GM control). With an average level of 2 g/kg, concentrations ranged from 1.5 to 2.5 g/kg (NK603) and from 1.7 to 2.3 g/kg (non-GM control). Ferulic acid levels in this maize therefore vary naturally by approximately 40%. In conclusion, the 16-30% difference described in the publication between the groups of feed corresponds to the 'natural' variability of this compound in maize.

The possible protective role (particularly in the liver) of ferulic acid (and numerous similar compounds) shown in a series of studies can be noted. For example, a protective effect has been found against mammary tumours induced by 7,12-dimethylbenz[*a*]anthrecene in Sprague Dawley rats (Baskaran *et al.* 2010). However, none of the scientific data currently available in the study by Séralini *et al.* support a protective role of dietary ferulic acid in rats or a supposedly harmful effect related to a 16-30% decrease in ferulic acid as observed in the study. More generally, it is difficult to assess the role of such a substance due to the multiple pleiotropic biological properties for which chemopreventive potential is often claimed with no solid epidemiological data.
To further study such assumptions on the effects of these natural substances, it would have been wise to have a comprehensive study on the composition of maize and diets (chow - pellet feed) containing the secondary metabolites that are commonly evaluated for maize.

[38] Refer to the 2005 joint AFSSA/AFSSAPS report "*Safety and benefits of dietary phyto-oestrogens – recommendations*", which can be viewed at the following address:
http://www.afssa.fr/Documents/NUT-Ra-Phytoestrogenes.pdf.
The recommendations are a maximum daily intake of 1 mg/kg body weight of isoflavone equivalents in humans.

Conclusion to section 3.3

In conclusion, after critically examining the relevant publications in the framework of this Request, the ECEAG notes the lack of publications involving long-term toxicological studies on formulated plant protection products and the limited number of publications on the long-term effects of GMPs. The publication by Séralini *et al.* (2012) combines these two approaches. Its major weakness is that in order to do so, it reduced the number of control groups and animals in each group. The results on mortality and tumour incidence are presented descriptively and are not statistically analysed. The authors thus make interpretations that are not supported by the study's data. The assumed mechanisms proposed by the team of Séralini *et al.* (2012) to explain the results were not confirmed by the ECEAG's analyses.

The two long-term studies on GMPs identified by the ECEAG (Malatesta *et al.* 2008; Sakamoto *et al.* 2008) do not offer evidence of GMP-related effects comparable to those described by Séralini *et al.* (2012) (onset of tumours and increased animal mortality). However, it should be noted that these study results cannot be fully applied to the Séralini study, since they were not conducted with maize but with glyphosate-tolerant soybeans, even though this tolerance was obtained through the synthesis of a CP4 EPSPS protein, like for NK603 maize. Furthermore, for Malatesta *et al.* (Malatesta *et al.* 2008), the study was conducted on a limited number of animals, a different species and only on female mice.

3.4 Conclusions drawn by the ECEAG

Séralini *et al.* (2012) conducted an ambitious study, employing considerable research resources, that was published in an internationally recognised food toxicology journal. This study is commendable for having addressed novel issues.

However, upon examination, the ECEAG experts consider that the authors' conclusions are not sufficiently supported by the data presented in the paper. Furthermore, the analysis provided by the ECEAG does not confirm the hypotheses on the mechanisms of action that the authors formulated to explain their results.

As a result, the ECEAG experts conclude that the results of the study as they have been published do not challenge the conclusions from previous risk assessments of NK603 maize and the use of ROUNDUP herbicide. This study cannot therefore be regarded as conclusive as to the potential health risk of food products derived from NK603 GM maize or of ROUNDUP.

Nevertheless, the ECEAG experts note the lack of studies on the potential effects of long-term exposure to various glyphosate-based formulations and the limited number of studies that have addressed the long-term effects of consuming GMOs.

Regarding the issue concerning revisions of GMO and plant protection product assessment principles , the ECEAG considers that it is too early to issue recommendations, which cannot in any case be based on a single study.

Regarding GMOs, the ECEAG experts note that there has been a gradual improvement in safety assessment criteria and standards; in particular, the strengthening of the substantial equivalence approach by implementing subchronic toxicity feeding studies on animals. However, whether current assessment methods can detect potential long-term effects and the plausibility of these effects are subjects of controversy in the scientific community. Given that there are so few studies documenting these effects, it is difficult to overcome this controversy. The ECEAG deems that these issues should be debated, especially in regard to the growing and foreseeable complexity (GM stacked events) of genetically-modified plants. The ECEAG experts therefore feel that it is necessary to deliberate further on whether the scientific principles for evaluating safety should be revised and this deliberation should be based on all the studies that have been conducted on a national level, particularly by ANSES, but also on an international level[39].

[39] In particular, current research projects (GRACE FP7-KBBE project, Project reference: 311957).

Regarding plant protection products, the regulations for placing these products on the market do not require long-term studies for commercial formulations. In particular, cumulative effects can be addressed using methods that are currently being developed in Europe. These methods are intended for application in studies on the cumulative effects of active substances present in the same formulation, particularly on exposed workers. The ECEAG experts consider that it would be appropriate to apply these methodologies to co-formulants, especially those, given their properties, for which toxicity reference values have been set. The experts also consider that more methodological research on the ‘cocktail effect' of formulations is needed.

4. AGENCY'S CONCLUSIONS AND RECOMMENDATIONS

ANSES endorses the conclusions and recommendations made by the ECEAG.

ANSES recalls its recent work on issues underlying risk assessment of GMOs and its methodological approaches for these assessments. Accordingly, as part of an innovative approach on the European level, ANSES issued an Opinion in 2011 recommending more rigorous conditions under which 90-day subchronic toxicity studies should be carried out, and proposed a very strict data analysis methodology. A draft European regulation is being finalised and was submitted to Member States in spring 2012; it requires that 90-day feeding studies be carried out using the conditions advocated by ANSES.

Regarding plant protection products, ANSES has actively participated in methodological developments at the European level to more effectively address the cumulative effects of active substances. These methods are currently being included in European safety assessment standards. They are intended for the study of cumulative effects of active substances and co-formulants.

Moreover, ANSES has put considerable effort into addressing the questions underlying the *Séralini et al.* study. Over the past few years, ANSES has carried out wide-reaching studies on endocrine disruptors and more generally on the issue of low doses. In addition, as part of the Périclés programme, ANSES research has also addressed the mixture effect of xenobiotics and the identification of their potential synergistic effects ("cocktail effects").

In general, the fact that publication of a study on the potential long-term effects of a GMO associated with a common plant protection product has sparked such an active public debate shows that more scientific knowledge is required in this area.
This debate is part of a wider scientific context that includes other diverse studies. On the one hand, there are studies funded by industry to meet regulations, and on the other hand, there is publicly-funded research, with more limited resources, that seeks to investigate potential health effects that have been little documented thus far. Although this situation is not specific to GMOs, GMOs attract considerable public attention and there is a particularly acute public desire for independent, objective research.

Thus, more generally speaking, ANSES calls for more public funding on the national and European levels for broad-scope studies to consolidate scientific knowledge on insufficiently documented health risks.

In light of the needs for studies and research highlighted by the ECEAG, ANSES recommends:

- more research on the potential health effects associated with the long-term consumption of GMOs or long-term exposure to plant protection products. This research should focus in particular on the issue of exposure to GMOs and to residues of associated plant protection preparations. These studies should be conducted using public funds and based on precise research protocols that address specific questions (investigated effects, monitored parameters, research methodology, number and nature of animals studied, complexity of the GMO, type of exposure, etc.). ANSES is prepared, along with other partners, and particularly other European health agencies, to work toward defining the general principles for these study protocols;
- and more broadly speaking, fostering research on the health issues associated with chronic exposure to xenobiotics (active substances, co-formulants), their mixtures and their potential interactions, especially regarding their effects when combined with GMOs.

The Director General

Marc Mortureux

KEY WORDS

GMOs, plant protection products, NK603, ROUNDUP, long-term study

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ANNEXES

Annex 1

Members of the emergency collective expert assessment group (NK603-R ECEAG)

Chairman

Mr Jean-Pierre CRAVEDI – Research Director, INRA, Toulouse

Members

Mr Michel GAUTIER – University professor, AGROCAMPUS OUEST, Rennes

Ms Mariette GERBER – Medical doctor– Researcher, INSERM, Retired

Mr Joël GUILLEMAIN – Toxicologist, Lecturer, University of Tours

Mr Florian GUILLOU – Research director, INRA, Nouzilly

Mr David MAKOWSKI – Research director, INRA, Thiverval-Grignon

Mr Fabrice NESSLANY – Department head for the Laboratory for Toxicology and Genetics, Institut Pasteur, Lille

Mr Erwan POUPON – University professor, Faculty of Pharmacy, University of Paris (Paris Sud)

Ms Marie-Anne ROBIN – Research director, INSERM, Rennes

Ms Paule VASSEUR – Professor Emeritus, University of Lorraine (Metz)

Annex 2

OECD standards for long-term studies and Good Laboratory Practice

It is important to distinguish between non-carcinogenic and carcinogenic chronic effects. Three OECD guidelines correspond to these effects:

- OECD 451 – Carcinogenicity studies

The purpose of long-term carcinogenicity studies is to observe test animals over most of their life span for the development of neoplastic lesions during or after exposure to various doses of a test substance by an appropriate route of administration. This guideline is intended primarily for use with rats and mice, and for oral administration. Both sexes should be used. Each dose group and concurrent control group should contain at least 50 animals of each sex. At least three dose levels and a concurrent control should be used. The test substance should be administered daily to animals via the oral route (or via dermal or inhalation administration) and the mode of exposure should be adjusted according to the toxicokinetic profile of the test substance. The duration of the study will normally be 24 months for rodents. For specific strains of mice, a duration of 18 months may be more appropriate. Termination of the study should be considered when the number of survivors in the lower dose groups or the control group falls below 25 percent. The results of these studies include measurements (weighing, food consumption), and, at least, daily and detailed observations, as well as gross necropsy and histopathology.

- OECD 452 – Chronic toxicity studies

The purpose of chronic toxicity studies is to characterise the profile of a substance in mammalian species (primarily rodents) following prolonged and repeated exposure. The guideline focuses on rodents and oral administration. Both sexes should be used. For rodents, at least 20 animals per sex per group should normally be used at each dose level, while for non-rodents a minimum of 4 animals per sex per group is recommended. At least three dose levels should be used in addition to the concurrent control group. Frequency of exposure is normally daily, but may vary according to the route chosen (oral, dermal or inhalation) and should be adjusted according to the toxicokinetic profile of the test substance. The duration of the exposure period should be 12 months. The study report should include measurements (weighing) and regular detailed observations (haematological examination, urinalysis, clinical chemistry), as well as necropsy procedures and histopathology.

- OECD 453 – Combined chronic toxicity/carcinogenicity studies

The objective of a combined chronic toxicity/carcinogenicity study is to identify carcinogenic and chronic effects in mammalian species, and to determine dose-response relationships following prolonged and repeated exposure. The rat is typically used for this study. For rodents, each dose group and concurrent control group intended for the carcinogenicity phase of the study should contain at least 50 animals of each sex, while for the chronic toxicity phase of the study they should contain at least 10 animals of each sex. At least three dose levels should be used, in addition to the concurrent control group for both the chronic toxicity phase and the carcinogenicity phase of the study. The three main routes of administration are oral, dermal, and inhalation. The guideline focuses on the oral route of administration. The duration of the study is normally 12 months for the chronic toxicity phase, and 24 months for the carcinogenicity phase. The study report should include measurements (weighing) and regular detailed observations (haematological examination, urinalysis, clinical chemistry), as well as necropsy procedures and histopathology. All these observations enable the detection of neoplastic effects and the determination of carcinogenic potential as well as general toxicity.

For active plant protection substances, the OECD 453 guideline is used in order to assess chronic and carcinogenic effects in a single study. A study in rats and a study in mice are required.

- Good Laboratory Practice (GLP)

Initially developed by the US Food and Drug Administration (FDA) in 1976 and then adopted by the OECD in 1978, the principles of Good Laboratory Practice (GLP) make up an organisational process that covers all organisational and operational aspects of the non-clinical safety testing of chemical products. Their objectives are to guarantee the quality, reproducibility and integrity of data generated for regulatory purposes so they may be accepted on an international level without duplicative testing.

For the European Union, GLP principles are defined in Directive 2004/10/EC. Study compliance with GLP principles is ensured through national programmes to verify studies and the inspection of testing laboratories. In Europe, the inspection and verification of Good Laboratory Practice are addressed in Directive 2004/9/EC.

The specific case of studies involving multiple sites is covered by special provisions (OECD ENV/JM/MONO(2002)9).

Annex 3

Prior assessments of ROUNDUP™ GT plus

Roundup GT Plus is the formulation that was used in the Séralini *et al.* (2012) study for administration in drinking water. The active ingredient in this formulation is glyphosate (in isopropylamine salt form).

Glyphosate is an active ingredient that was approved for use in Europe in 2001 and reference values were set at that time. The EU is currently reassessing the safety of glyphosate. Germany is the Rapporteur Member State and is responsible for reviewing all the regulatory toxicological data and the data found in the literature. This reassessment started in May 2012 and will be made available to EFSA[40] and the other Member States in June 2013.

In the assessment report on glyphosate and its addendum, the following studies, submitted by the many notifiers employing this active ingredient, were reviewed by the German authorities. This assessment report was also reviewed by experts in other Member States.

Glyphosate as the active ingredient:

- kinetics (absorption, distribution, metabolism, elimination): 12 studies, including 3 from the literature on glyphosate or ROUNDUP.
- acute oral toxicity in rats, mice: more than 20 studies.
- acute dermal toxicity in rats and rabbits: 15 studies.
- acute toxicity by inhalation in rats: 9 studies.
- skin irritation in rabbits: 12 studies.
- eye irritation in rabbits: 11 studies.
- skin sensitisation in guinea pigs: 9 studies.
- subacute oral toxicity: in rats (3 studies over 28 days), mice (1 study over 30 days) and dogs (2 'range-finding' studies).
- subchronic oral toxicity: 9 studies in rats (90 days) including one from the literature conducted by the NTP[41], 3 studies in mice (90 days) including one from the literature conducted by the NTP, 6 studies in dogs (durations of 3 months to 1 year).
- subacute dermal toxicity: 3 studies in rabbits (21 or 28 days), 1 study in rats (21 days).
- subacute toxicity by inhalation: 1 14-day study in rats and 1 literature review on 28-day studies in rats with ROUNDUP.
- *in vitro* genotoxicity studies: 9 Ames tests, 2 chromosomal aberrations assays, 1 genetic mutation test on mammal cells, 6 DNA repair tests.
- *in vivo* genotoxicity studies: 3 micronucleus or chromosomal aberration assays in rats or mice, 3 germ cell tests (dominant lethal tests).

[40] EFSA: European Food Safety Authority
[41] NTP: National Toxicology Program
http://ntpsearch.niehs.nih.gov/query.html?qt=glyphosate&col=015abst&col=020rpt&charset=iso-8859-1

The studies show that glyphosate does not have any genotoxic properties *in vivo*.

- Chronic and carcinogenesis studies: 4 studies in rats and 4 studies in mice, summarised below:

Species/Duration	Doses	NOEL[42]/NOAEL[43]	Target organ	Reference
Wistar Rat/2 years 50 rats of each sex in each group.	0 – 100 – 1000 – 10,000 ppm	NOAEL: 1000 ppm (60 mg/kg/day) NOEL: 100 ppm (6.3 mg/kg/day)	Liver damage (biochemical indications). Cataracts (weak evidence) These effects are observed at 10,000 ppm (LOAEL). At 1000 ppm (LOEL), the observed effects on alkaline phosphatase were not consistent throughout the study.	Suresh, 1996
Sprague Dawley rats/2 years 85 rats of each sex in each group.	0 – 10 – 100 – 300 mg/kg/day	NOEL: 10 mg/kg/day	Salivary glands (histological effects). Mild hepatic toxicity These effects are observed from 100 mg/kg/day.	Atkinson *et al.*, 1993
Sprague Dawley rats/2 years 60 rats of each sex in each group.	0 – 2000 – 8000 – 20,000 ppm	NOEL: 2000 ppm (89 mg/kg/day)	Cataracts, mild liver damage at 20,000 ppm. Gastric inflammation at 8000 ppm (LOEL).	Stout and Ruecker, 1990
Sprague Dawley rats/26 months 50 rats of each sex in each group.	0 – 3 – 10 – 31 mg/kg/day in male rats. 0 – 3.4 – 11 – 34 mg/kg/day in female rats.	NOEL: 31 mg/kg/day	No treatment effects observed.	Lankas, 1981
CD-1 mice/2 years 50 mice of each sex in each group.	0 – 100 – 300 – 1000 mg/kg/day	NOAEL: 1000 mg/kg/day	No treatment effects observed.	Atkinson *et al.*, 1993
Balb/c mice/18 months 25 mice of each sex in each group.	0 – 75 – 150 – 300 ppm	NOAEL: 150 ppm (15 mg/kg/day)	Decrease in weight gain and in food consumption at 300 ppm.	Bhide, 1988 *
CD-1 mice/2 years 50 mice of each sex in each group.	0 – 1000 – 5000 – 30,000 ppm	NOEL: 1000 ppm (157 mg/kg/day)	Liver damage (30,000 ppm) and bladder damage (5000 ppm) observed only in males.	Knezevich and Hogan, 1983
CFLP/LATI mice/18 months 50 mice of each sex in each group.	0 – 100 – 300 ppm	NOAEL: 300 ppm (30 mg/kg/day)	No treatment effects observed.	Vereczkey and Csanyi, 1982 (rev. 1992)

*Study not appropriate for evaluating carcinogenic effects.

None of these studies show any significant increase in the incidence of tumours in animals treated with glyphosate.

[42] NOEL: No observed effect level
[43] NOAEL: No observed adverse effect level

- Studies on reproductive functions: 2 studies on one generation in rats, 3 studies on two generations in rats, 3 studies on three generations in rats, 1 literature study of the specific effects on fertility.
- Developmental toxicity studies: 5 studies in rats, 5 studies in rabbits and 1 study in mice.

The results of these studies show that glyphosate does not lead to any alteration in reproductive functions.

Studies have also been carried out on AMPA[44], which is the main metabolite of glyphosate:

- Acute toxicity by three exposure routes, dermal irritation and eye irritation, dermal sensitisation, subacute and subchronic studies (4 studies in rats for 14 to 90 days, 2 studies in dogs for 1 month and 90 days). There are also 3 studies on developmental toxicity.

Based on these studies, the following reference values (expressed in dose of active ingredient) were derived:

- ADI: 0.3 mg/kg/day
- ARfD: not applicable
- AOEL: 0.2 mg/kg/day

Based on these figures, the hazard classifications of glyphosate and its salts were determined by a European expert group for classification and labelling.
The harmonised European classifications according to Regulation (EC) no. 1272/2008 are the following:

For glyphosate acid:
H318 Eye Irritant Cat. 1 (R41 in the former classification system)
H411 Aquatic Chronic 2 (R51/53 in the former classification system)

For glyphosate salts:
H411 Aquatic Chronic 2 (R51/53 in the former classification system)

Glyphosate-based formulations
Fourteen different preparations were studied in the EU assessment report. For most of these formulations, the required data were provided: acute toxicity by three administration routes, dermal irritation and eye irritation, dermal sensitisation, dermal absorption (*in vitro* on human and monkey skin, *in vivo* in monkeys).
*In vitro g*enotoxicity studies have also been carried out with formulations containing glyphosate and a surfactant (Williams 2000[45]): 3 Ames tests, 2 tests on *Drosophila*, 1 chromosomal aberration assay, 2 sister chromatid exchange tests.
Some *in vivo* genotoxicity studies are also available: 6 micronucleus tests. There are also 3 tests on DNA effects.
The results do not indicate that Roundup formulations have genotoxic properties.

Co-formulants used in ROUNDUP formulations
A summary document from the US EPA (2009)[46] reviews the main toxicological studies available on ROUNDUP co-formulants. These co-formulants have also been assessed by ANSES as adjuvants in herbicide sprays.
The toxicological dossiers of these substances include acute toxicity studies, studies on irritation, sensitisation, *in vitro* genotoxicity studies (Ames tests, mutagenicity and chromosomal aberrations),

[44] AMPA: aminomethylphosphonic acid
[45] Williams GM*, et al.* (2000) Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans. *Regulatory Toxicology and Pharmacology* **31**(2 I), 117-165
[46] US-EPA proposed the following TRVs (source: http://www.epa.gov/fedrgstr/EPA-PEST/2009/June/Day-17/p14113.pdf)

subchronic toxicity studies for 90 days in rats (2 studies) and in dogs (1 study), 2 screening studies for reproductive toxicity properties in rats.

By cross-examining the data available for other adjuvants considered to be equivalent, the reference toxicological values were determined for these adjuvants:

- ADI: 0.15 mg/kg/day
- ARfD: 0.72 mg/kg
- AOEL: 0.15 mg/kg/day

Chemical formulation of ROUNDUP GT Plus

The formulation of ROUNDUP GT Plus contains isopropylamine salt of glyphosate, a co-formulant and water. AFSSA assessed this formulation in 2006 for use in home gardens as a weed-killer before planting, the use intended by the manufacturer.

The results relating to human health risks presented in the AFSSA opinion of 16 April 2007 are as follows:

Regarding toxicological properties

The acceptable daily intake (ADI) of glyphosate acid is 0.3 mg/kg/day, a value that was set when glyphosate was included in Annex I of Directive 91/414/EEC. This ADI was derived by applying a safety factor of 100 to the no-effect dose obtained in a two-year study of oral administration in rats.

Other studies carried out with comparable formulations, containing the same co-formulant and 490 g/L of glyphosate instead of 450 g/L in the ROUNDUP GT Plus formulation gave the following results:

- an LD_{50}[47] by the oral route and dermal route in rats of more than 5000 mg/kg,
- mild eye irritation in rabbits,
- no skin irritation in rabbits,
- no skin sensitisation in guinea pigs.

Given these results and the data available on co-formulants, this formulation does not require any hazard classification with regard to its acute toxicity or its irritant or sensitisation potential.

Regarding data with regard to operator, bystander and worker exposure

The acceptable operator exposure level for glyphosate acid, set when it was included in Annex I of Directive 91/414/EEC, is 0.2 mg/kg/day. This AOEL was derived by applying a safety factor of 100 to the no-effect dose obtained from an oral teratogenicity study in rabbits. The level of dermal absorption used for the operator exposure assessment is 3% (determined from an *in vitro* study on human skin and an *in vivo* study in Rhesus monkeys).

In consideration of the conditions in which ROUNDUP GT Plus is applied in home gardens, without gloves, the systemic operator exposure was estimated from specific studies available using the following parameters:

- application dose: 5.6 mL/10m², with 460 g/L glyphosate and 898 g/L co-formulant;
- application method: spray application with a pre-pressurised aerosol can.

[47] LD_{50}: The median lethal dose (lethal dose, 50%) is a statistical value of the dose of a substance or formulation at which a single administration by the oral route causes death in 50% of the treated animals.

The estimated exposure, expressed as a percentage of the AOEL is as follows:

% AOEL glyphosate	% AOEL co-formulant
35	31

In light of these results, the health risk to home garden users without protective gloves is considered to be acceptable[48] during preparation and application of the formulation.

An additional risk assessment to account for potential long-term cumulative effects with regard to the presence of several substances in a mixture can be performed.

Various approaches for assessing cumulative exposure risks are described in the literature. The approach described below is based on that advocated by the Chemical Regulation Directorate (CRD UK) and on that presented in ANSES's report of June 2010[49].

The methodology[50] used is based on calculating risk quotients (RQ) defined for each active ingredient as the ratio of estimated exposure levels to the reference value (AOEL). The sum of the risk quotients (Σ RQ) for each substance is then calculated to determine the risk index (RI).
If the RI is <1 then the risks for the operator, bystanders and workers are considered acceptable.
If the RI is >1 then the risks for the operator, bystanders and workers are considered unacceptable.

The % of AOEL, the RQs for each active ingredient as well as the RIs are as follows:

% AOEL [Risk quotients (RQ)]		Sum of risk quotients or risk indices
Glyphosate 0.2 mg/kg/day	Co-formulant 0.15 mg/kg/day	
35% (0.35)	31% (0.31)	0.66

The exposure of home gardeners (without gloves) is less than 100% of the AOEL for glyphosate and the co-formulant.

The RI estimating the cumulative risk of active ingredients in the formulation is <1 (0.66). The risk due to the simultaneous exposure to glyphosate and the co-formulant can therefore be considered as acceptable.

Regarding the data on residues and consumer exposure
The intended use does not lead to any direct exposure of crops when the formulation is sprayed. However, given the systemic properties of glyphosate (translocation within the plant) a risk assessment is necessary. This assessment considers the level of absorption of the substance by subsequent crops planted in the treated area.

Available data on the active ingredient
There have been studies on metabolism in the main categories of plants (23 types of crop) and in animals (goats and layer hens) as well as studies on the processing of plant products and residues in subsequent crops. These studies show that glyphosate can be included as a potential residue found in plant- and animal-derived products. These results were used for the risk assessment.

[48] Commission Regulation (EU) No 546/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards uniform principles for evaluation and authorisation of plant protection products

[49] Proposal of a methodology for assessing the aggregated and cumulative health risks associated with exposure to a mixture of benzylbutylphthalate and dibutyl phthalate. Expert Committee on Assessment of Risks associated with Chemical Substances, June 2010, final version no. 1, www.afsset.fr

[50] An information note is available on the ANSES website.

Consumer risk assessment for glyphosate

Based on the ADI of 0.3 mg/kg bw/day, the assessment of consumer exposure[51] shows that for an adult, toddlers (13-18 months) and infants (7-12 months), the theoretical maximum daily intake (TMDI) estimated from the maximum residue levels (MRLs) determined for products of plant and animal origin represents less than 18% of the ADI. The chronic risk for all consumers together is considered as acceptable.
For the various intended uses, the intervals before harvesting have been set to ensure that the level of residues in food is lower than the MRLs.

Consumer risk assessment for the co-formulant

The intended use does not lead to any direct crop exposure when the formulation is sprayed. Given the data reported in the literature, it appears that the co-formulant does not have systemic properties and it stays on the plant where it was applied. Its metabolites, in particular C14 fatty acid (myristic acid) can migrate in plants but it is considered to be of low toxicity and, as part of a European assessment of fatty acids (C7-C20), it has not been deemed necessary to set toxicological reference values for them. In addition, studies found in the scientific literature on the degradation of this type of co-formulant in the environment[52,53,54] show that these substances are rapidly broken down by microorganisms. Moreover, the high Koc[55] values indicate that the co-formulant binds strongly to soil when coming into contact with it. Based on these data, it is estimated that the absorption by roots of the co-formulant in the ROUNDUP GT Plus formulation in plants that are growing after treatment is negligible. It is therefore highly unlikely that this co-formulant would leach into groundwater or that it could be found there.

In conclusion, under conditions of intended use, consumer exposure to the co-formulant is considered negligible. Therefore, the cumulative risk assessment of glyphosate and the co-formulant, which could be conducted using the methodology indicated above or using the one currently being developed by the EU (European ACROPOLIS programme), does not appear to be warranted.

The risk to consumers for the intended use can be considered as acceptable.

Glyphosate (or ROUNDUP) with NK603

The French Food Safety Agency issued an opinion on 9 March 2010 regarding a marketing authorisation application from Monsanto Agriculture France SAS for the glyphosate-based ROUNDUP READY formulation for use as a weedkiller on maize crops (only on glyphosate-tolerant maize with the NK603 transformation event and expressing the CP4 EPSPS protein).
Residue trials carried out using the ROUNDUP READY formulation on glyphosate-tolerant maize crops were submitted and examined as part of the application process.
The operator, consumer and environmental risks assessed were all deemed acceptable. The Opinion[56] is available for consultation on the ANSES website.

[51] PRIMo revision 2 (EFSA, 2007). Reasoned opinion on the potential chronic and acute risk to consumers' health arising from proposed temporary EU MRLs according to Regulation (EC) No 396/2005 on Maximum Residue Levels of Pesticides in Food and Feed of Plant and Animal Origin. 15 March 2007.

[52] Behaviour of three nonionic surfactants following foliar application - Peter J. Holloway, Dawn Silcox - Department of Agricultural Sciences, University of Bristol, Long Ashton Research Station, Long Ashton, Bristol, BS18 9AF, UK.- British Crop Protection Conferences – weeds(1985).

[53] Behavior of Polyoxyethylene sorbitan ^{14}C-monooleate in Tobacco and kidney bean leaves.-Yukio Sugimura and Tsuneyyuki Takeno -J. Pesticide Sci 10, 323-239(1985).

[54] Behavior and fate of ethoxylated alkyl phenol nonionic surfactant in barley plants - Gary E Stolzenberg, Prudence A Olson, Richard G Zaylstie, Eugene Mansager - J Agri Food Chem (1982) 637-644.

[55] Koc: soil organic carbon-water partition coefficient: ratio of the mass of a chemical that is adsorbed in the soil per unit mass of organic carbon in the soil.

[56] AFSSA Opinion No 2007-3111 of 9 March 2010 on a marketing authorisation application for the glyphosate-based formulation ROUNDUP READY, submitted by Monsanto Agriculture France SAS.

Annex 4

Prior assessments of NK 603 maize

The NK603 event confers glyphosate tolerance to maize. The genes introduced into this maize come from a common soil bacterium, *Agrobacterium sp.* strain *CP4.* The gene construct used contains two genes that are inserted in tandem into a single insertion site, allowing for the expression of two 5-enolpyruvylshikimate-3-phosphate synthase enzymes: CP4 EPSPS and CP4 EPSPS L214P. One gene is regulated by the rice actin promoter and the other by the cauliflower mosaic virus 35S promoter. The two proteins differ only by one amino acid substitution of proline for leucine at position 214 (CP4 EPSPS L214P).
EPSPS proteins are enzymes involved in the shikimic acid metabolic pathway (Annex 7), a route used by plants, fungi and micro-organisms for the biosynthesis of aromatic amino acids (phenylalanine, tyrosine and tryptophan). These proteins are ubiquitous in these organisms but not in animals, which do not produce their own aromatic amino acids and have to obtain them from food.

Glyphosate is an herbicide that acts in the step catalysed by the EPSPS protein by blocking the synthesis of the three aromatic amino acids. In susceptible plants, this blockage subsequently prevents the synthesis of proteins, auxin and lignin, deregulates the chorismate pathway and ultimately leads to plant death.
Two transgenes derived from *Agrobacterium sp.* CP4 are used in NK603 genetically-modified maize, resulting in the synthesis of the bacterial EPSPS protein, which is less susceptible to glyphosate and allows the maize to synthesise aromatic amino acids (Padgette *et al.*, 1996). Maize containing the NK603 event is therefore tolerant to glyphosate at doses used for the control of susceptible weeds.

NK603 maize has been the subject of two AFSSA Opinions for feed (AFSSA, 2003 and 2004) and two for food (AFSSA, 2003 and 2004); having requested additional documents from the applicant, AFSSA did not make a decision until January 2004. EFSA issued a favourable Opinion on 25 November 2003 (EFSA, 2003). NK603 maize authorisations have been issued based on two European regulations: Directive 2001/18/EC for animal feed and Regulation 258/97/EC for human food.
The European Commission has published two decisions authorising the marketing of foods and food ingredients derived from the genetically modified maize line NK603 as novel foods or novel food ingredients[57] and for animal feed[58].

In 2006, a new marketing authorisation application of the genetically modified glyphosate tolerant maize NK603 for cultivation, food and feed uses and import and processing was submitted under Regulation (EC) no. 1829/2003. It did not contain any information that had not already been included in those applications previously examined by AFSSA when assessing the health risks related to consumption of this maize in humans and animals, apart from presenting studies assessing the environmental risks related to its cultivation. These studies, which did not fall within AFSSA's scope of expertise, were assessed by the French Biomolecular Engineering Commission (CGB). EFSA issued an Opinion on 27 May 2009 on both the framework of Regulation (EC) no. 1829/2003 and the renewal of the authorisation for NK603 maize for products authorised in the former regulation (EFSA, 2009). AFSSA's opinion was not sought for the renewal of the marketing authorisation for this maize.

The applications assessed by AFSSA for NK603 maize (Requests 2003-SA-0027, 2003-SA-0047, 2003-SA-0401 and 2003-SA-0242) contained information related to:

[57] Commission Decision of 3 March 2005 (2005/448/EC) authorising the placing on the market of foods and food ingredients derived from genetically modified maize line NK 603 as novel foods or novel food ingredients under Regulation 258/97/EC (OJEU 21/06/05).
[58] Commission Decision of 19 July 2004 (2004/643/EC) concerning the placing on the market, in accordance with Directive 2001/18/EC, of a maize product (*Zea mays* L. line NK603) genetically modified for glyphosate tolerance (OJEU 18.09.04).

- the genetic modification and molecular characterization of NK 603 genetically modified maize,
- the chemical composition of grain maize and the whole plant and its nutritional qualities,
- CP4 EPSPS and CP4 EPSPS L214P protein levels in maize tissues,
- an assessment of the toxic potential of the CP4 EPSPS and CP4 EPSPS L214P proteins through acute toxicity studies[59], *in vitro* degradation experiments and searches for sequence homology with toxic and allergenic proteins,
- a nutritional value study in growing chickens,
- a 90-day feeding toxicity study undertaken in rats (see following paragraph) and calculations of margins of exposure for maize.

The allergenic potential of NK603 maize was assessed in light of a number of points as recommended in the guidelines:

- the lack of known allergenic potential for the source organism (*Agrobacterium*),
- the lack of protein sequence identity (including for eight consecutive amino acids) between the primary structures of the CP4 EPSPS and CP4 EPSPS L214P proteins and those of known allergenic and toxic proteins,
- rapid *in vitro* hydrolysis of the CP4 EPSPS and CP4 EPSPS L214P proteins.

With regard to these points, these two proteins and NK603 maize had no suspected allergenic potential.

Subchronic toxicity study

A 90-day subchronic toxicity study was undertaken in 2001 in rats of both sexes (20 rats of each sex/treatment) to examine the effects of a diet containing two incorporation rates (11 and 33%) for NK603 grain maize compared to a diet containing maize with the same genetic base and six other maize varieties. The maize was treated with glyphosate. This study did not show any differences regarded by experts as relevant for any of the observed biological parameters, between the control rats and those fed the diets containing GM maize (AFSSA, 2003).

In December 2009, AFSSA issued an internal Request to analyse the results of a publication by Spiroux de Vendômois *et al*. (Spiroux de Vendômois *et al.,* 2009) which re-examined the data of this subchronic toxicity study. This publication showed significant differences in certain groups and treatments for liver and kidney function. In its Opinion 2009-SA-0322, AFSSA assessed modified parameters in treated and control groups and considered that these heterogeneous variations, which were unrelated, were a perfect example of the lack of correlation between statistically significant variations and their biological relevance.

Bibliography for Annex 4

AFSSA 2003-SA-0047, Avis de l'Agence française de sécurité sanitaire des aliments relatif à un dossier d'autorisation de la mise sur le marché d'un maïs génétiquement modifié tolérant au Roundup Ready lignée NK 603 en vue de son utilisation comme tout autre maïs, à l'exclusion de la culture, sur le territoire de l'Union européenne, au titre de la directive 2001/18/CE, le 7 mars 2003 http://www.anses.fr/Documents/BIOT2003sa0047.pdf

AFSSA 2003-SA-0242, Examen des compléments d'information en réponse aux objections des Etats membres relatifs à un dossier d'autorisation de la mise sur le marché d'un maïs génétiquement modifié tolérant au Roundup Ready lignée NK 603 en vue de son utilisation comme tout autre maïs, à l'exclusion de la culture, sur le territoire de l'Union européenne, au titre de la directive 2001/18/CE2003-SA-0242, le 5 janvier 2004.
http://www.anses.fr/Documents/BIOT2003sa0242.pdf

AFSSA 2003-SA-0027, Avis de l'Agence française de sécurité sanitaire des aliments relatif au rapport d'évaluation initiale établi par les autorités néerlandaises concernant la mise sur le marché

[59] No toxic effects were found at doses above 572 mg/kg bw for CP4EPSPS and 817 mg/kg bw for CP4EPSPS L214P (single-dose acute toxicity in mice)

de grains et de produits dérivés de maïs de la lignée NK 603 résistant au glyphosate (Roundup Ready) au titre du règlement 258/97, le 21 février 2003.
http://www.anses.fr/Documents/BIOT2003sa0027.pdf

AFSSA 2003-SA-0401 Examen des compléments d'information en réponse aux objections des Etats membres relatifs à un dossier d'autorisation de la mise sur le marché de grains et de produits dérivés de grains de maïs génétiquement modifié tolérant au Roundup Ready lignée NK 603 au titre du règlement (CE) n°258/97, le 13 janvier 2004
http://www.anses.fr/Documents/BIOT2003sa0401.pdf

AFSSA 2009-SA-0322 Avis de l'Agence française de sécurité sanitaire des aliments relatif à son auto-saisine sur l'article publié dans 'International Journal of Biological Sciences' et intitulé 'A comparison of the effects of three GM corn varieties on mammalian health, le 5 février 2010.
http://www.anses.fr/Documents/BIOT2009sa0322.pdf

EFSA, 2003 Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the safety of foods and food ingredients derived from herbicide-tolerant genetically modified maize NK603, for which a request for placing on the market was submitted under Article 4 of the Novel Food Regulation (EC) No 258/97 by Monsanto1 Opinion adopted on 25 November 2003 The EFSA Journal (2003) 9, 1-14

EFSA, 2009 Scientific Opinion of the Panel on Genetically Modified Organisms on applications (EFSA-GMONL-2005-22 and EFSA-GMO-RX-NK603) for the placing on the market of the genetically modified glyphosate tolerant maize NK603 for cultivation, food and feed uses and import and processing, and for renewal of the authorisation of maize NK603 as existing product. The EFSA Journal (2009) 1137, 1-50.

Padgette et al, 1996 The composition of glyphosate-tolerant soybean seeds is equivalent to that of conventional soybeans. *J Nutr.* 126(3):702-16.

Spiroux de Vendômois J. S., Roullier F., Cellier D., Séralini, G. E. 2009 A comparison of the effects of three GM corn varieties on mammalian health. *International Journal of Biological Sciences* **5**(7), 706-726.

Annex 5

List of publications relative to 90-day subchronic oral toxicity studies, conducted in the context of risk assessments for GMPs

References and title	GMP	Newly expressed proteins	Tested material and incorporation rates	Treatments	Rats strain Number of rats/treatment group/sex	Results
Appenzeller *et al.*, 2008 Subchronic feeding study of herbicide-tolerant soybean DP-356Ø43-5 in Sprague-Dawley rats. Food and Chem. Tox. 46 2201-2213.	HT genetically modified Soybean DP-356043-5	GAT GM-HRA	Toasted meal (20% w/w) and ground hulls (1.5% w/w).	6 groups: -GM soybean 356043 -GM soybean 356043 treated intended herbicides -near-isoline control, -3 non transgenic commercial varieties	Sprague Dawley 12	No adverse effects
Appenzeller et al., 2009a Subchronic feeding study with GM stacked trait lepidopteran and coleopteran resistant (DAS-1507-1xDAS-59122-7) maize grain. Food and Chem. Tox. 47 1512-1520.	HT and IR genetically modified Maize 1507x59122	Cry1F PAT Cry1Ab34 Cry1Ab35 PAT	Maize grain (34%w/w)	6 groups: -GM maize 1507x59122 - near-isoline control - 3 non transgenic commercial varieties	Sprague Dawley 12	Grain from 1507x59122 maize is as safe and nutritious as that obtained from non-GM maize
Appenzeller et al., 2009b Subchronic feeding study of grain from herbicide tolerant maize DP-98140-6 in Sprague Dawley rats. Food and Chem. Tox. 47 2269-2280.	HT genetically modified Maize 98140	GAT	Maize grain (35 and 38% w/w)	6 groups: -GM maize 98140 -GM maize 98140 treated intended herbicide - near-isoline control - 3 non transgenic commercial varieties	Sprague Dawley 12	No adverse health effects
Dryzga et al., 2007 Evaluation of the safety and nutritional equivalence of a GM cottonseed meal in a 90 day dietary toxicity study in rats. Food and Chem. Tox. 45 1994-2004.	IR and HT genetically modified cotton	Cry1F Cry1Ac PAT	Meals (10% concentration)	5 groups -GM Widestrike -near-isoline control -3 non transgenic commercial varieties	Sprague Dawley 12	Lack of any toxicity of GM Widestrike cottonseed

References and title	GMP	Newly expressed proteins	Tested material and incorporation rates	Treatments	Rats strain Number of rats/treatment group/sex	Results
Hammond et al., 2004 Results of a 13 week safety assurance study with rats fed grain from glyphosate tolerant corn. Food and Chem. Tox. 42 1003-1014.	HT genetically modified maize NK603	CP4 EPSPS	Maize grain (11 and 33%)	10 groups -11 or 33% GM maize -11 or 33% near isoline control -6 non transgenic commercial varieties 33%	Sprague Dawley 20	This study confirms ROUNDUP ready corn grains is as safe and nutritious as existing commercial corn hybrids.
(Hammond, Dudek et al. 2006) Results of a 90-day safety assurance study with rats fed grain from corn borer-protected corn. Food and Chem. Tox. 44 1092-1099	IR genetically modified maize MON810	Cry1Ab	Maize grain (11 and 33%)	10 groups -11 or 33% GM maize -11 or 33% near isoline control -6 non transgenic commercial varieties 33%	Sprague Dawley 20	MON810 is considered to be substantially equivalent to, and as safe and nutritious as, conventional corn varieties.
(Hammond, Dudek et al. 2006) Results of a 90-day safety assurance study with rats fed grain from rootworm-protected corn. Food and Chem. Tox. 44 147-160	IR genetically modified maize MON863	Cry3Bb1 NptII	Maize grain (11 and 33%)	10 groups -11 or 33% GM maize -11 or 33% near isoline control -6 non transgenic commercial varieties 33%	Sprague Dawley 20	MON863 is considered to be substantially equivalent to, and as safe and nutritious as, conventional corn varieties.
(Hammond, Lemen et al. 2008) Safety assessment of SDA soybean oil: Results of a 28-day study and a 90-day/one generation reproduction feeding study in rats. Regul. Tox. and Pharmacol. 52, 311-323.	SDA genetically modified soybean (rich in stearidonic-acid)	Δ6 and Δ15 desaturases	Soybean oil 4 g/kg body weight	4 groups -4g or 1.5g SDA soybean oil (GM) -4g near isogenic control soybean oil -4 g menhaden oil	Sprague Dawley 25	The result of the 90 day/one generation reproduction feeding study found no evidence of treatment related adverse effects up to the highest dosages of SDA soybean oil tested.
(He, Huang et al. 2008) Comparison of grain from corn rootworm resistant T DAS -59122-7 maize with non-T maize grain in a 90 day feeding study in Sprague Dawley rats. Food and Chem. Toxicol. 46 1994-2002.	IR and HT genetically modified maize DAS-59122	Cry34Ab1 Cry35Ab1 PAT	Maize flour (50% and 70%)	5 groups -50 or 70% GM maize 59122 -50 or 70% near isoline control maize -43.3% maize flour (=control diet)	Sprague Dawley 10	The results demonstrated that it was as safe and nutritious as non-transgenic maize grain
(He, Tang et al. 2009) A 90-day toxicology study of transgenic lysine-rich maize grain (Y642) in Sprague Dawley rats, Food and Chem. Toxicol. 47 425-432.	Lysine-rich genetically modified maize (Y642)	sb401 (a gene from potatoes)	Maize grain (30 and 76%)	5 groups: -30% and 76% GM Y642 -30% and 76% near isoline control maize -43.3% maize flour (=control diet)	Sprague Dawley 10	The results demonstrated that Y642 is as safe and nutritious as conventional non-GM maize grain

References and title	GMP	Newly expressed proteins	Tested material and incorporation rates	Treatments	Rats strain Number of rats/treatment group/sex	Results
(Healy, Hammond et al. 2008) Results of a 13-week safety assurance study with rats fed grain from corn rootworm-protected, glyphosate-tolerant MON87017 corn. Food and chem. Toxicol. 46 2517-2524.	IR and HT genetically modified Optimum GAT maize MON87017	Cry3Bb1 CP4EPSPS	Grain (11 and 33%)	9 groups: -11% and 33% GM MON87017 -11% and 33% near isogenic control -6 non transgenic commercial varieties 33%	Sprague Dawley 20	No adverse health effects were detected in rats following 13 weeks of dietary exposure to grain from genetically modified Optimum GAT maize.
(Malley, Everds et al. 2007) Subchronic feeding study of DAS-59122-7 maize grain in Spague-Dawley rats, Food and Chem. Tox. 45 1277-1292.	IR and HT genetically modified maize DAS-59122	Cry34Ab1 Cry35Ab1 PAT	Grain (35%)	5 groups -35% GM maize DAS-59122 - 35% non transgenic near isogenic control 1 variété commerciale - - 2 non transgenic commercial varieties 35%	Sprague Dawley 12	Results from the current study demonstrated that 59122 maize grain is as nutritious and wholesome as conventional maize grain when evaluated in a subchronic feeding study in rats.
(Poulsen, Kroghsbo et al. 2007) A 90-day safety study in Wistar rats fed genetically modified rice expressing snowdrop lectin Galanthus nivalis (GNA) Food and Chem. Tox. 45 350-363.	Genetically modified Rice GNA (expressing lectin galanthus nivalis)	GNA lectin galanthus nivalis	Rice flour	2 groups: 60% GM rice GNA 60% control parental rice	Wistar 16	In the present study several differences were observed between rats fed diets with GM and parental rice. Most of these differences appeared to be related to the increased water intake of the rats fed GM rice, which probably relates to the GNA lectin content, but none of the effects were considered to be adverse.
Liu et al. 2012 A 90-day subchronic feeding study of genetically modified maize expressing Cry1Ac-M protein in Sprague-Dawley rats. Food and Chem. Tox. 50 3215-3221.	IR genetically modified maize	Cry1Ac_M	Maize	7 groups 12.5, 25, 50% GM maize 12.5, 25, 50% non GM maize Maize commercial line	Sprague Dawley 10	Safe as conventional
(Schroder, Poulsen et al. 2007) A 90-day safety study of genetically modified rice expression Cry1Ab protein (Bacillus thuringiensis toxin) in Wistar rats. Food and Chem. Tox. 45 339-349.	IR genetically resistant Rice (KMD1)	Cry1Ab	Rice flour	2 groups: 60% GM rice KDM1 60% non-transgenic parental wild type rice	Wistar 16	The results show no adverse or toxic effects of KDM1 rice when tested in the design used in this 90-day study.

References and title	GMP	Newly expressed proteins	Tested material and incorporation rates	Treatments	Rats strain Number of rats/treatment group/sex	Results
(Wang, Wang et al. 2002) Toxicological evaluation of transgenic rice flour with a synthetic *cry1Ab* gene from bacillus thuringiensis. J. Sci. Food Agric. 82 738-744.	IR genetically resistant Rice (KMD1)	Cry1Ab	Rice flour	4 groups: 64% GM rice KDM1 32% GM rice KDM1 16% GM rice KDM1 64% non-transgenic parental wild type rice	Sprague Dawley 10	KDM1 rice flour was safe to rats in general.
Zhu et al. 2012 A 90-day feeding study of glyphosate-tolerant maize with the G2-aroA gene in Sprague-Dawley rats. Food and Chem Tox 18. S0278-6915	HT genetically modified maize	G2-AroA gene	Maize	7 groups 12.5, 25, 50% GM maize 12.5, 25, 50% non GM maize Maize commercial line	Sprague Dawley 10	Safe and nutritious
Zhou et al 2011 A 90-day toxicology study of high-amylose transgenic rice grain in Sprague-Dawley rats. Food Chem Tox, 2011 49(12):3112-3118.	High amylose	RNAi	Rice	3 groups 70% GM 70% isogenic Control diet	Sprague Dawley	as safe as the conventional non-transgenic rice for rat consumption

IR: Insect resistant
HT: Herbicide tolerant

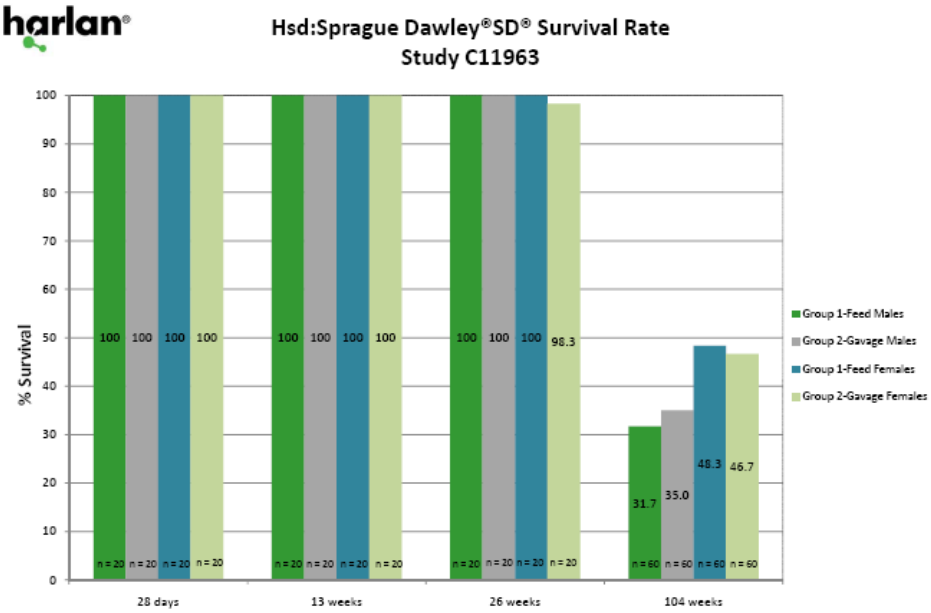
Annex 6

Comparison between data in the literature on mortality rates and incidences of tumours, and the study by Séralini *et al.* (2012)

Mortality (Figure 1 and Table 1)

Table 1 and Figure 1 show the mortality rates described in the literature for Sprague Dawley rats, the strain used in the experiment by Séralini *et al.* (2012) (Chandra *et al.* 1992; Dinse *et al.* 2010; Nakazawa *et al.* 2001; Prejean *et al.* 1973). Séralini *et al.* (2012) observed two early deaths (males: 100 days 11% GMO and 120 days 22% GMO + R) for which the exact causes are not given. The literature reports a few rare cases of the onset of mammary tumours before 140 days (Kuzutani, 2012).

Figure 1: Mortality in Sprague Dawley rats from Harlan Laboratories, which supplied the rats for the study by Séralini *et al*. (2012).



Incidence of tumours (Tables 2, 3, 4 and 5)

In females, the literature describes a high incidence of mammary and pituitary tumours. This corresponds to the observations reported by Séralini *et al*. (2012).

However, in males, several studies show a high incidence of testicular, dermal and adrenal tumours (Chandra, Riley 1992, Nakazawa 2001). The article by Séralini *et al*. (2012) does not mention tumours in these organs. The incidence of liver and kidney tumours is very low in Sprague Dawley rats (Chandra, Riley 1992, Nakazawa 2001). It is difficult to assess these results and compare them to the observations made by Séralini *et al*. (2012). Indeed, the article by Séralini *et al*. (2012) mentions pathologies of the liver and kidneys without specifying whether or not they are tumour-related.

Table 1: Percentage of mortalities in Sprague Dawley rats

	Prejean (1973) (77 weeks or 540 days) 180 rats/sex	Nakazawa (2001) Groups A and B 120/sex	Chandra (1992) 2 years 1340 males 1329 females	Iffa Crédo (105 weeks or 2 years) 100 rats/sex	Dinse (2010) 2 years 330 females
Males	33%	A 11% B 24%	51%	50%	
Females	58%	A 58% B 65%	54%	35%	28-51%

Table 2: Frequency in % of tumours observed in Sprague Dawley rats in publications by Nakazawa (2001) and Chandra (1992)

	Sprague-Dawley Shizuoka Japan Nakazawa *et al.* 2001			Sprague-Dawley Charles River Chandra *et al.* 1992		
Organs	**Male (240)**	**Female (240)**	**Total (480)**	**Male (1340)**	**Female (1329)**	**Total (2669)**
Liver	3.33 (8)	2.08 (5)	5.41 (13)	2.6(35)	0.83 (11)	1.7 (46)
Pituitary gland	36.67 (88)	69.59 (167)	53 (255)	27.8 (373)	49.4 (659)	38.6 (1032)
Mammary gland	–	32.92 (79)	32.92 (79)	1.5 (20)	31.6 (420)	
Kidneys	1.25 (3)	0.83 (2)	1 (5)	0.97 (13)	0.45 (6)	0.7 (19)
Adrenal gland	12.92 (31)	10 (24)	11.4 (55)	6.1 (82)	3 (40)	4.6 (122)

Table 3: Frequency in % of tumours observed in Sprague Dawley rats and Swiss mice from the publication by Prejean *et al.* 1973.
(Size of groups) Analysis at 540 days

	Sprague-Dawley Charles River Prejean *et al.* (1973)			Swiss mice Charles River Prejean *et al.* (1973)		
Organs	**Male (179)**	**Female (181)**	**Total (360)**	**Male (101)**	**Female (153)**	**Total (254)**
Liver	0	0	0	2 (2)	0	0.8 (2)
Pituitary gland	16.2 (29)	29.3 (53)	22.8 (82)	0	1.9 (3)	1.2 (3)
Mammary gland	2.2 (4)	32 (58)	17.2 (62)	1 (1)	1.9 (3)	1.6 (4)
Kidneys	0	0	0	2 (2)	0	0.8 (2)
Adrenal gland	7.8 (14)	10 (18)	8.8 (32)	1 (1)	0	0.4 (1)

Table 4: Frequency in % of tumours observed in Sprague Dawley rats and Fisher 344/N rats in publications by Dinse *et al.* (2010) and Brix *et al.* (2005)

(Size of groups) Analysis at 2 years

	Sprague-Dawley Harlan Dinse *et al.* (2010)	Sprague-Dawley Harlan Brix *et al.* (2005)	Fischer 344/N Harlan Dinse *et al.* (2010)
Organs	**Females (473)**	**Females (371)**	**Females (450)**
Liver	1.27 (4)	1.3 (5)	0.89 (4)
Pituitary gland	40.55 (191)	42.3 (157)	45.77 (206)
Mammary gland	80.1 (379)	85.2 (316)	52.88 (238)
Kidneys	1.47 (7)	0.6 (2)	0.22 (1)
Adrenal gland	9.35 (44)	1.1 (4)	3.78 (17)

Table 5: Incidence in % of the onset of tumours in the mammary gland in Sprague Dawley rats. % (number of animals concerned/total number of animals)

Mammary gland			
	Male	**Female**	**Origin**
Okada *et al.* (1981)		20.3 (13/64)* 75.8 (72/95)**	JCL Japan
Chandra *et al.* (1992)	1.5 (20/1340)	31.6 (420/1329)	Charles River
Nakazawa *et al.* (2001)		32.9 (79 /240)	Japan SLC
Brix *et al.* (2005)		85.2 (316/371)	Harlan
Dinse *et al.* (2010)		80.1 (379/473)	Harlan
Séralini *et al.* (2012)		50 (5/10)	Harlan
Harlan (2012)	3.4 (4/~120)	91.6 (109/~120)	Harlan

*420 days; ** - 756 days

Bibliography for Annex 6

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Kuzutani K, *et al.* (2012) Spontaneous Mammary Adenocarcinoma in a Twelve-week-old Female Sprague-Dawley Rat. *J Toxicol Pathol* **25**(3), 221-4.

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Annex 7

Metabolic pathway of shikimic acid

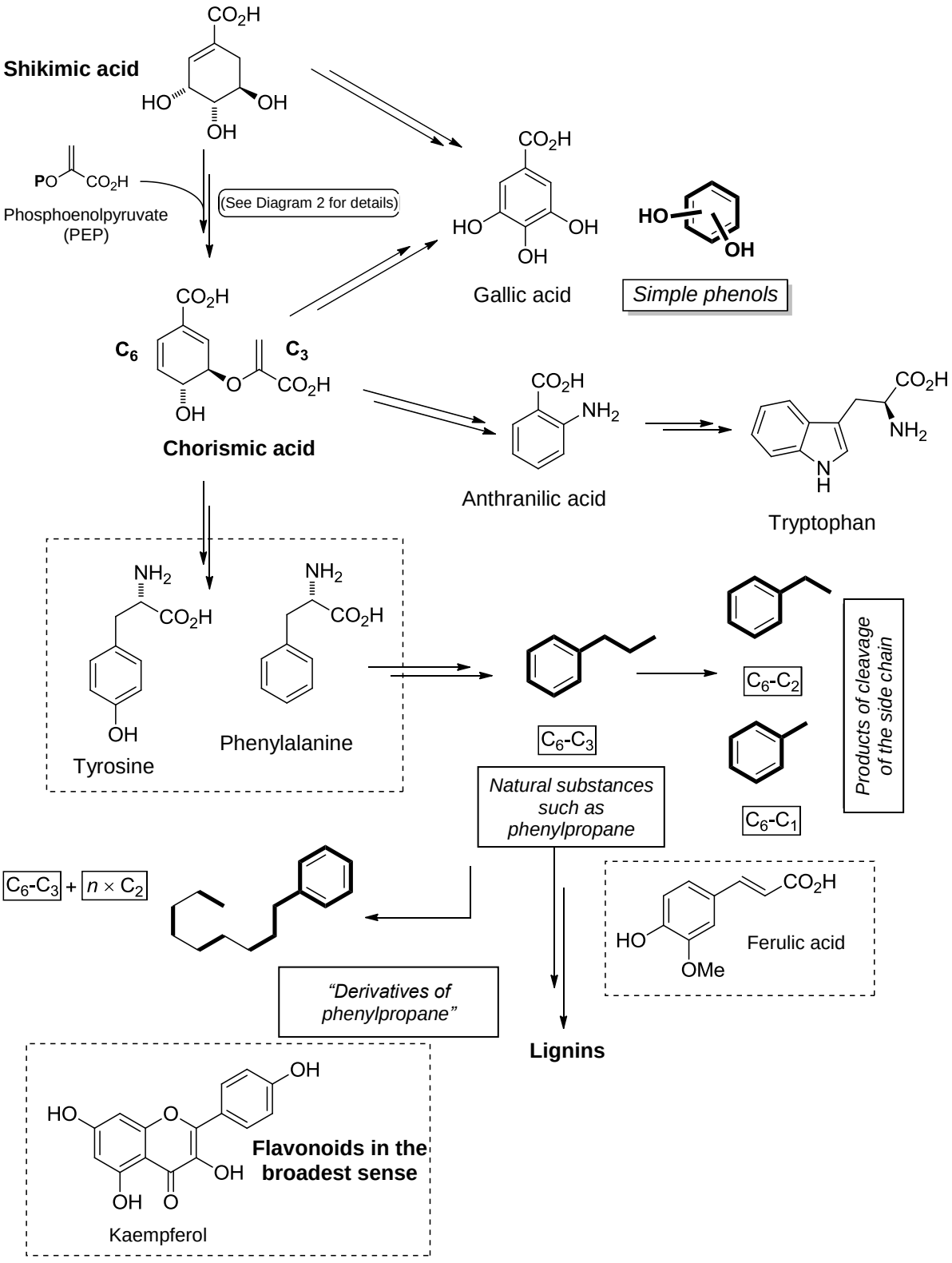

Diagram 1 – Shikimic acid pathway

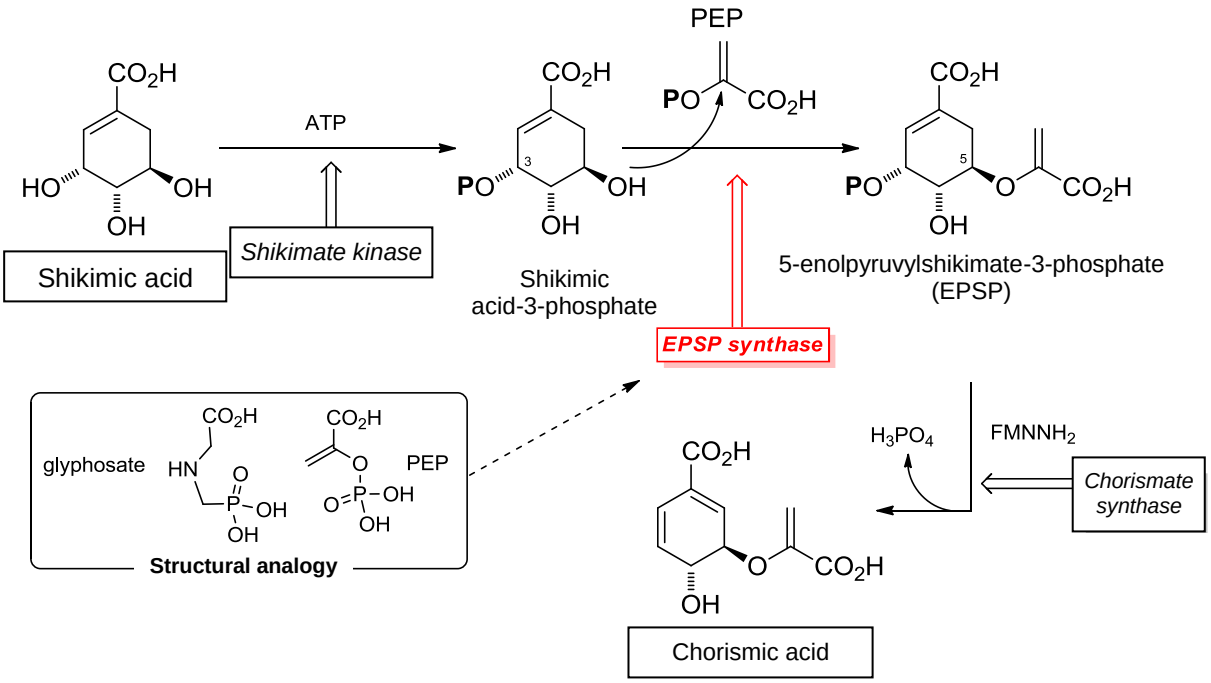

Diagram 2 – Position of EPSP synthase in the metabolism of shikimic acid