European Food Safety Authority

EFSA Journal 2011;9(4):1930

SCIENTIFIC OPINION

Scientific Opinion on the Chemical Safety of Irradiation of Food[1]

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Irradiation (gamma rays, electrons or X-rays) is applied to foods for improving food safety and removing and reducing pests that are harmful to plants or plant products. As ionising radiation passes through food, it creates a trail of chemical transformations by primary and secondary radiolysis effects. The main reported radiolytic products are certain hydrocarbons and 2-alkylcyclobutanones produced from the major fatty acids in food, and some cholesterol oxides and furans. Most of these substances are also formed in food that has been subjected to other processing treatments and are thus not exclusively formed by irradiation. Furthermore, the quantities in which they occur in irradiated food are not significantly higher than those being formed in heat treatments. Since the last SCF opinion in 2003, several *in vitro* genotoxicity studies on irradiated foods have been published. The available data indicate that at least some 2-alkylcyclobutanones may induce DNA damage *in vitro*. No *in vivo* genotoxicity studies are available; however, a genotoxic hazard in humans is considered unlikely by the Panel in view of the plausible indirect mechanism underlying the genotoxicity of alkylcyclobutanones *in vitro*. Concerning other radiolytic products no new relevant toxicological studies have been reported. The weight of evidence from recent literature regarding biological effects supports the food classes and radiation doses specified in previous SCF opinion in 2003. The only new contrary evidence was indicated in publications on leukoencephalomyelopathy in cats which have been fed mainly or exclusively with highly irradiated feed. However a clear mechanistic explanation in terms of risk assessment has not been established. Considering that only a very limited quantity of food is irradiated in Europe currently, the Panel is of the view that there is not an immediate cause for concern. However, the relevance of the cats studies for human health should be clarified.

KEY WORDS

Irradiation, chemical food safety, radiolytic products, 2-alkylcyclobutanone, Hydrocarbon, radiation dose.

---

1 On request from the European Commission, Question No EFSA-Q-2006-034, adopted on 25 November 2010.

2 Panel members: Arturo Anadón, Mona-Lise Binderup, Wilfried Bursch, Laurence Castle, Riccardo Crebelli, Karl-Heinz Engel, Roland Franz, Nathalie Gontard, Thomas Haertlé, Trine Husøy, Klaus-Dieter Jany, Catherine Leclercq, Jean-Claude Lhuguenot, Wim C. Mennes, Maria Rosaria Milana, Karla Pfaff, Kettil Svensson, Fidel Toldrá, Rosemary Waring, Detlef Wölfle. Correspondence: cef-unit@efsa.europa.eu

3 Acknowledgement: The Panel wishes to thank the members of the Working Group on Irradiation of food (chemical safety): Arturo Anadón, Carl Blackburn, Laurence Castle, Klaus-Dieter Jany, Christophe Nguyen-The, Georg A. Schreiber, Mario Stahl, Eileen M. Stewart, Kettil Svensson and Fidel Toldrá.

Suggested citation: EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on the Chemical Safety of Food Irradiation. EFSA Journal 2011;9(4):1930. [57 pp.] doi:10.2903/j.efsa.2011.1930. Available online: www.efsa.europa.eu/efsajournal.htm

SUMMARY

Following a request from the European Commission, the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) was asked to deliver a scientific opinion on the chemical safety of irradiation of food taking into account the latest available scientific information.

For this task an extensive literature search was performed covering relevant scientific publications until December 2009 for evaluation. The literature database was maintained and updated until May 2010.

Irradiation is applied to foods for various purposes such as improving food safety and removing and reducing pests that are harmful to plants or plant products.

Food irradiation involves exposing food to ionizing radiation under controlled conditions. The types of ionizing radiation used are as follows: i) Gamma rays as emitted by the radionuclides cobalt-60 (Co-60) or caesium-137 (Cs-137); ii) Electrons (electron beams, E-beams) or iii) X-rays. As ionising radiation passes through food it creates a trail of chemical transformations by primary and secondary radiolysis effects. The main reported radiolytic products are certain hydrocarbons and 2-alkylcyclobutanones produced from the major fatty acids in food, and some cholesterol oxides and furans. Most of these substances are also formed in food that has been subjected to other processing treatments and are thus not exclusively formed by irradiation. Furthermore, the quantities in which they occur in irradiated food were not significantly higher than those being formed by heat treatments. Until recently 2-alkylcyclobutanones were only reported in irradiated food, thereby indicating that they may be unique radiolytic products. Recently one publication reported the occurrence of some 2-alkylcyclobutanones in commercial non-irradiated fresh cashew nuts and nutmeg samples. As no further evidence of the natural existence of 2-alkylcyclobutanones has been reported, it would be pertinent to treat these findings with some caution until the results are validated by further experimental work.

EU legislation requests that any irradiated food or food ingredient, even if it is present in trace amounts in a non-irradiated compound food, has to be labelled as “irradiated” or “treated with ionizing radiation” in order to allow consumers an informed choice. Analytical methods to detect radiation treatment have been validated and standardised by the European Committee for Standardisation (CEN) and later accepted as Codex General Methods. Since publication of the standard methods, further developments have been made regarding the sensitivity and the range of foods which can be analysed. However, these results would have to be validated before they can be standardised.

The irradiation-induced changes in food components are generally small and not significantly different from those reported in other conventional preservation processes, especially those based on thermal treatment. The changes in some components that are sensitive to irradiation, like some vitamins, may be minimised by using proper treatment conditions.

The Panel considered the various terms used for defining radiation dose. When food is irradiated there are three main definitions of dose that can be stated; the maximum dose, the minimum dose and the overall average dose. The maximum dose and minimum dose can be measured directly. In contrast, the overall average dose is not a processing parameter but is a concept once used in Codex standards, and still found in national standards and legislation (*e.g.,* Directive 1999/2/EC). Since 2003, the Codex Standard no longer uses the concept of overall average dose but instead uses the minimum and maximum dose. The Panel considers this approach sensible, as the overall average dose cannot be measured directly but only estimated.

Currently radiation doses are assigned to general food classes and specific food commodities. The Panel recognises the shortcomings of the current classification as it does not consider the physical state of the food (*e.g.*, fresh or frozen food of the same class, water activity), the different composition

of products within a food class (*e.g.*, fat content) and the diversity of food products nowadays available to the consumer (*e.g.*, ready-to-eat foods, sliced meat or cheese).

When considering the literature since 2003, the Panel found that even though there is a large number of publications on food irradiation, the number relevant for evaluating chemical safety is limited. Most of the reported studies investigate the toxicological properties of radiolytic products, mainly 2-alkylcyclobutanones.

Since the last SCF Opinion in 2003, several *in vitro* genotoxicity studies (Ames test, chromosome aberrations in mammalian cells, micronucleus test and SOS chromo test) on irradiated foods have been published.

The available data indicate that at least some 2-alkylcyclobutanones may induce DNA damage *in vitro*. No *in vivo* genotoxicity studies are available; however, a genotoxic hazard in humans is considered unlikely by the Panel in view of the plausible indirect mechanism underlying the genotoxicity of alkylcyclobutanones *in vitro*.

Concerning other radiolytic products no new relevant toxicological studies (genotoxic, subchronic, carcinogenic/chronic, reproduction) have been reported.

The weight of evidence from the existing literature, arising from the recent survey of literature regarding biological effects, supports the food classes and radiation doses specified in previous SCF opinions. The only new contrary evidence was indicated in publications on leukoencephalomyelopathy (LEM) in cats which have been fed mainly or exclusively with highly irradiated feed (>25 kGy). This finding has only been reported with cats. In one report, dogs consumed the same pet food and did not show the disease. Several hypotheses have been put forward in the literature (*e.g.*, specific sensitivity of cats to deficiency in vitamins which may be caused by irradiation, peroxides generated by irradiation). However a clear mechanistic explanation in terms of risk assessment has not been established.

Based on studies reviewed, no conclusion can be drawn for relevance to humans. Due to the lack of data, it cannot be possible to make a link between the cat LEM disease and possible appearance of this disease in humans where LEM pathophysiology has not been established yet, remaining uncertainties. Information on the cause and pathogenesis in cats should be collected, including data on the relationship between irradiation dose, composition of feed, the amount of consumed irradiated feed and the elicitation of the leukoencephalomyelopathy.

It is noted that these neurological effects seen in cats (feed irradiated in the range from 25.7 to 53.6 kGy) were observed at doses that exceeded the normal dose range (1-10 kGy) for food for human consumption. Furthermore, in the experiments cats were entirely fed with an irradiated diet (min dose: 25 KGy) but this is not the case for humans as our diet varies more widely.

Considering that only a very limited quantity of food is irradiated in Europe currently, the Panel is of the view that there is not an immediate cause for concern. However, the relevance of the cats studies for human health should be clarified.

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The Scientific Committee on Food (SCF) expressed opinions on irradiated foods in 1986, 1992, 1998 and 2003 and gave favourable opinions on irradiation of a number of foodstuffs for which the classes and maximum doses have been listed.

Irradiated foods are regulated by Directive 1999/2/EC[4], which covers general and technical aspects for carrying out the process, labelling of irradiated foods and conditions for authorising food irradiation. In addition, Directive 1999/3/EC[5] establishes a Community list of food and food ingredients authorised for treatment with ionising radiation. So far, this list contains only a single food category: dried aromatic herbs, spices and vegetable seasonings. The list should still be completed.

Until a more comprehensive positive list enters into force, Member States may maintain existing national authorisations provided that the foodstuffs concerned have been subject to a favourable opinion of the SCF, and the average absorbed doses do not exceed the recommended limit values.

The Commission is now considering different options for drawing up a proposal to complete the positive list of Directive 1999/3/EC. Any possible addition to this list will have to be considered in the light of an update of the scientific opinions previously expressed by SCF and the other criteria laid down in the legislation.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Commission asks the EFSA to issue an opinion on the safety of the irradiation of certain food products within 9 months from the date or receipt of this request. Using the previous opinions of the Scientific Committee on Food on irradiation of food, the EFSA should establish whether the food classes and doses specified in these opinions are still up-to-date to ensure consumer safety.

Clarification of the terms of reference

After receiving the mandate it was clarified with the Commission that the request concerned the food classes and radiation doses listed in the table enclosed to the mandate and concerning the food classes evaluated as acceptable for irradiation with corresponding radiation doses by the SCF opinions expressed in 1986, 1992 and 1998 (see also Table 1 in chapter 1.2).

Furthermore it was clarified that the Commission would like to be assured that the safety assessment of food irradiation takes into account the latest available scientific information and would therefore be grateful that EFSA does the necessary review.

Finally, a new deadline for the delivery of the opinions was agreed (31 December 2010).

[4] Directive 1999/2/EC of the European Parliament and of the Council of 22 February 1999 on the approximation of the laws of the Member States concerning foods and food ingredients treated with ionising radiation. OJ L 66, 13.03.1999, p.16-22.

[5] Directive 1999/3/EC of the European Parliament and of the Council of 22 February 1999 on the establishment of a Community list of foods and food ingredients treated with ionising radiation. OJ L 66, 13.3.1999, p 24-25.

ASSESSMENT

1. Preamble

1.1. Understanding of the mandate and scope of the opinion

Regarding the request from the European Commission the EFSA Panel on food contact materials, enzymes, flavourings and processing aids (CEF) took into consideration new information published in the scientific literature since the last previous opinions of the Scientific Committee. For this purpose a literature search was commissioned and the relevant scientific articles were screened. This opinion considers the chemical safety and nutritional aspects of irradiated food. Although food packaging is treated with radiation, this aspect is not considered in the present opinion.

In a separate opinion, the EFSA Panel on Biological hazards (BIOHAZ) deals with the efficacy of irradiation, understood as the ability of irradiation to reduce food-borne pathogens in food, and with the microbiological safety of irradiation, understood as the contribution of irradiation to reduce the risk to human health from food-borne pathogens. The BIOHAZ Panel also considers potential microbiological risks linked to food irradiation, such as the development of resistance, the possibility that irradiation might be used to mask unhygienic food production practices.

1.2. Previous Reports/Opinions of the Scientific Committee on Food (SCF)

In 1986 the SCF was requested by the European Commission to provide advice on the wholesomeness of irradiated food. A report was provided by the SCF (SCF, 1986) concluding as follows:

"*On the basis of all available evidence, the Committee recommends that in the context of an overall assessment of the wholesomeness of irradiated foods only those specific irradiation doses and food classes should be endorsed that are indicated as appropriate, not only from a strict toxicological point of view, but also from a chemical, nutritional and technological standpoint. […] The Committee believes that the health significance of any changes which may take place in the listed foods at the indicated radiation doses is not different from the health significance of the changes which are induced by heat treatment.*

The Committee sees, in principle, no objection to considering an extension of the list to other applications provided that appropriate information is given for evaluation following the criteria considered in the present report."

In 1992, a second request was made to the SCF in order to consider whether Camembert cheese could be added to the food classes listed in the previous report for which irradiation was considered acceptable. The SCF (SCF, 1992) extended the conclusions formulated in 1986 and concluded that "*the treatment of Camembert cheeses manufactured from raw milk with gamma-radiation at doses up to 2.5 kGy was acceptable from a health point of view*".

The SCF was then consulted a third time in order to evaluate the potential risks to public health arising from the treatment with ionising radiation of eight foodstuffs submitted by the authorities of a Member State. The opinion (SCF, 1998) reported that "*the Committee accepts that irradiation of these eight food products does not pose a risk to public health. The Committee has no objections to add the submitted 8 food items to the already published list of acceptable irradiated food classes at the overall average radiation doses requested in the submissions for technological reasons*".

As reported in the citation above, it has to be emphasised that on the occasion of the opinion adopted by SCF in 1998, the basis of the request for assessing some of the food commodities was the use of irradiation for technical purposes, rather than for microbiological safety purposes.

The food classes/commodities and radiation doses evaluated by the abovementioned SCF Reports/Opinions as acceptable from a public health standpoint are reported in Table 1.

Table 1. General food classes and specific food commodities and radiation doses* evaluated as acceptable by the SCF

Food class/commodity assessed by the SCF	Overall average radiation dose (kGy)	Doses (kGy)
Fruits [(a)]	Up to 2	
Vegetables [(a)]	Up to 1	
Cereals [(a)]	Up to 1	
Starchy tubers [(a)]	Up to 0.2	
Spices & condiments[(a)]	Up to 10	
Fish & shellfish [(a)]	Up to 3	
Fresh meats [(a)]	Up to 2	
Poultry [(a)]	Up to 7	
Camembert cheeses manufactured from raw milk [(b)]		Up to 2.5
Frog's legs [(c)]	Up to 5	
Shrimps [(c)]		5
Gum arabic [(c)]		3
Casein / caseinates [(c)]		Up to 6
Egg white [(c)]		Up to 3
Cereal flakes [(c)]		10
Rice flour [(c)]		Up to 4
Blood products [(c)]	10	

(a): SCF (1986)
(b): SCF (1992)
(c): SCF (1998)

*As expressed by SCF (see comments in section 2.3.5)

Questions in relation to food irradiation were posed to the SCF on two further occasions.

In 2002 the SCF published a statement (SCF, 2002), following a request to assess the results of a report to evaluate the toxicological properties of 2-alkylcyclobutanones (2-ACB), known to arise from irradiation-induced scission of triglycerides in irradiated fat-containing foods. The SCF concluded that "*as the adverse effects noted refer almost entirely to in vitro studies, it is not appropriate, on the basis of these results, to make a risk assessment for human health associated with the consumption of 2-ACBs present in irradiated fat-containing foods*".

Subsequently, the SCF was asked in 2003 to revise its report delivered in 1986 and in particular to advise whether it was appropriate to specify a maximum dose for the treatment of certain products and whether it was appropriate to evaluate foodstuffs individually taking into account a number of aspects (safety of irradiated foods for the health of consumers, technical needs, no substitute for good hygiene and good manufacturing and agricultural practices, the need to specify conditions for high dose irradiation). The SCF was asked also as a follow up on the conclusions of a Joint FAO/IAEA/WHO Study Group on high-dose irradiation (WHO, 1999), which concluded that the data on radiation chemistry, toxicology, microbiology and nutritional properties of foods treated with radiation doses above 10 kGy were adequate. This Study Group further concluded that food irradiated to any dose appropriate to achieve the intended technical objective was both safe to consume and nutritionally adequate (WHO, 1999).

On that occasion the SCF (SCF, 2003) concluded that "*as regards the microbiology of foods irradiated with doses above 10 kGy essentially the same issues arise as with any other accepted non-sterilizing food processing method and no additional hazards to health arise from the use of irradiation*". However, due to the existing toxicological studies, "*it is not possible for the Committee to accept at present the suggested removal of the upper limit of 10 kGy for the production of safe and wholesome irradiated foods. The Committee would be prepared to reconsider its position, when a more adequate database for the evaluation of the safety and wholesomeness of foodstuffs irradiated at doses above 10 kGy has been provided. In addition, the Committee would wish to consider the need for achieving an advantageous technological purpose by the irradiation of foods with doses above 10 kGy. At present, the only technological need recognised by the Committee would be the decontamination by irradiation of spices, dried herbs and vegetable seasonings, where doses up to 30 kGy may be needed to ensure a product in a satisfactory hygienic condition. On the basis of the information presently supplied to it, the Committee is still of the opinion that it is appropriate to specify a maximum dose for the treatment of certain food products by ionising radiation and that irradiated foodstuffs should continue to be evaluated individually taking into account the technological need and their safety*".

2. Introduction

Foods are irradiated for various purposes such as improving food safety and removing and reducing pests that are harmful to plants or plant products. Directive 1999/2/EC specifically allows food to be irradiated for the following purposes:

- to reduce the incidence of food-borne disease by destroying pathogenic organisms,
- to reduce spoilage of foodstuffs by retarding or arresting decay processes and destroying spoilage organisms,
- to reduce loss of foodstuffs by premature ripening, germination or sprouting,
- to rid foodstuffs of organisms harmful to plant or plant products (phytosanitary treatment).

Modern food safety management systems rely on a farm to fork approach and involve a range of actions at each step of the food chain. In particular, good hygienic practices, good manufacturing practices and HACCP principles must be applied, throughout the entire food chain (as laid down by Regulations (EC) No 852/2004[6] and 853/2004[7]).

Methods to inactivate micro-organisms (heat or chemical treatments) present on or in foods usually cause some modifications in the characteristic of the food. In particular, food irradiation has been investigated as a method which could inactivate micro-organisms while causing fewer changes in sensory characteristics.

2.1. Literature search/methodology

The starting point of the literature research was a database of the Max Rubner Institute (MRI), formerly Federal Research Center of Food and Nutrition, in Germany. Since 1955, food irradiation data (scientific publications, patents, etc.) were collected, analysed and indexed. With more than

[6] Corrigendum to Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. OJ L 139, 30 April 2004, p. 1-19.
[7] Corrigendum to Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. OJ L 139 of 30 April 2004, p 1-61.

17,000 entries (status: 2008), this database is one of the most comprehensive on food irradiation worldwide.

In addition, a further literature database was provided by the UK Food Standards Agency, Project A05009, undertaken by the Cardiff School of Health Sciences, Wales (Fielding, 2008). Also, additional sources of information were sought by a literature search executed including the SCOPUS and Web of Knowledge scientific abstracts (Web of Science, Food Science and Technology Abstracts (FSTA), CAB Abstracts, Biological Abstracts).

Field unit configuration and clean up (typing errors, typos, and duplicates) was conducted in the existing databases to minimize non-conformities. In accordance with the task, the resulting database was restricted to literature which was published between the years 1986 and October 2009.

Subsequently, the literature in the resulting database was sorted corresponding to the areas of the task, depending on the relevant appearance of the keywords "food" and " irradiation", in combination with "safety", "nutrients", appearance of "effic" for "efficacy" in the title, abstract or keyword-list of the respective entry.

After the merger of databases by the EFSA secretariat, it was maintained and updated until May 2010, in order to support the working group members with the relevant original publications.

2.2. Food irradiation technologies

Irradiation technology is used around the world to sterilize medical devices and pharmaceuticals, preserve artefacts, process cosmetics, packaging and food, and enable material improvements in consumer and manufactured goods. Although there are facilities dedicated to one purpose (*e.g.*, medical device sterilization), most are multipurpose facilities and irradiate a wide range of different products. Usually, irradiation is offered as a service by a contract irradiator, who takes delivery, processes the product to the desired level and then returns the treated goods.

2.2.1. Sources

Food irradiation involves exposing food to ionizing radiation under controlled conditions. The following types of ionizing radiations are covered by international standards of the Joint FAO/WHO Codex Alimentarius Commission and are therefore internationally recognized for the treatment of foods and permissible under Directive 1999/2/EC concerning foods and food ingredients treated with ionising radiation;

1. Gamma rays with energies of 1.17 and 1.33 MeV[8] as emitted by the radionuclide cobalt-60 (Co-60) or gamma rays of 0.66 MeV as emitted by caesium-137 (Cs-137);
2. Electrons (electron beams, E-beams) generated from machine sources operated at or below an energy level of 10 MeV; or
3. X-rays generated from machine sources operated at or below an energy level of 5 MeV.

None of the radiation techniques described induces at the specified energy levels measurable amounts of radioactivity (Diehl, 1995; Terry and McColl, 1992; WHO, 1994), therefore the natural content of radioactivity of food is not affected by these ionizing radiations.

Gamma rays, E-beams and X-rays have different properties and therefore present different technological benefits and disadvantages. However, each type of ionizing radiation initially interacts with food nutrients to produce similar reactive chemical intermediates that are transient and dissipate

[8] The mega electron volt (MeV) is a unit of energy, 1 MeV is equal to 1.6×10^{-13} joules.

rapidly after exposure to ionizing radiation. The effects of irradiation are mainly due to the indirect action of these transient chemicals rather than by the direct effect of the radiation itself. A given dose of gamma, E-beam or X-ray radiation gives rise to a similar effect (*e.g.*, equivalent doses of gamma or E-beam or X-ray ionizing radiation can be used to inactivate pathogenic and spoilage organisms; retard or arrest decay processes; prevent premature ripening, germination or sprouting, and rid foodstuffs of organisms harmful to plants or plant products).

The vast majority of food irradiation facilities are gamma irradiation facilities. For example, 27 of the 33 food irradiation facilities (23 in the EU and 10 worldwide) approved in 2010 to irradiate food for sale in the European Union are Co-60 gamma facilities and six are E-beam facilities. Food irradiation has developed in many countries and although the use of electron beams is increasing, and there is interest in using X-rays to treat foods, it is likely that the majority of commercial food irradiation facilities will continue to be gamma irradiation facilities for a considerable time (Kume *et al.*, 2009).

2.2.2. Gamma irradiation facilities

Gamma rays are emitted spontaneously during the radioactive decay of Co-60 and Cs-137. Most gamma irradiation facilities use Co-60, a radioactive metal that decays with a half-life of 5.3 years (every 5.3 years the amount of Co-60 will half in value). Few, if any, commercial gamma facilities use Cs-137 as a gamma ray source. Although it has a longer half-life of 30.1 years (and is therefore potentially more economically attractive as it takes longer to decay), Cs-137 emits gamma rays that are approximately half the energy of those emitted by Co-60 and under normal conditions caesium exists as an anion in a chemically stable ionic salt. The metallic form of Co-60 and its higher gamma ray energies offer considerable technological advantages for large irradiation facilities.

Part of the normal operating procedure for a gamma food irradiation facility, is to increase the duration of time that food is exposed to ionizing radiation, as the gamma ray source gets older. This is necessary in order to compensate for radioactive decay and with Co-60 this can be achieved by a monthly adjustment of a few percent to slow the speed that food travels around the radiation source. The radioactive source has to be replenished from time to time in order to augment the source and maintain an acceptable processing throughput. The gamma radiation cannot be switched off and the facility is operated around the clock in order to make full use of the radioactive material.

2.2.3. E-beam facilities

E-beams are produced by accelerating a stream of electrons; they are generated by machines and not by radioactive material. The high energy electrons are focused into a narrow beam-spot and this spot of incident electrons is scanned across food as it is travels perpendicular to the beam direction, through the irradiator. E-beams deliver dose at a high rate, and in less than a second can impart a dose that would take hours a gamma irradiation facility to deliver. In contrast to gamma facilities, E-beams can be switched off if necessary. However, E-beams do not penetrate into food to as great a depth as gamma rays and are therefore not as suitable for treating large bulk packages of food.

2.2.4. X-ray facilities

X-rays are also generated by machines and can be switched off if necessary. Here electrons are accelerated at a metallic target (*e.g.*, tantalum, tungsten or gold) and this generates a stream of X-rays. The process is not efficient, much of the E-beam energy is lost as heat, but the X-ray conversion efficiency increases with increasing atomic number of the metallic target material and with increasing incident E-beam energy. At present, very few foods are irradiated by X-rays. There is at least one commercial X-ray facility that has irradiated fruits, however it is not specified whether this facility is approved to irradiate food for sale in the European Union (Follett, 2004). Nevertheless, X-ray irradiation is finding more favour in association with E-beams, other radiation processing applications

as they are more penetrating than E-beams, making it possible to process large bulk packages but without the need for radioactive material. It is likely that X-ray irradiation will become more wide spread in future as the technology advances.

2.3. Understanding the dose

2.3.1 Dosimetry and absorbed radiation dose

Dosimetry is the measurement of absorbed radiation dose (commonly referred simply to as dose). Dose has units of Gray (Gy). 1 Gy is equal to the transfer of 1 joule of energy per kilogram of material (food). Food irradiation generally utilizes treatment doses of thousands of gray (kilogray, kGy). 1 kGy is equal to 1000 joules per kilogram (of food).

Dosimeters are used to record dose. When they are exposed to ionizing radiation, dosimeters undergo a change in physical or chemical properties that can be measured. This change is proportional to dose and dose rate, and it can be calibrated to a recognized dose standard. For example, plastic film containing a dye that changes colour in proportion to dose is the basis of a commonly used dosimetry system. The colour change can be measured accurately using a spectrophotometer and the degree of radiation inducing colour change can be calibrated to a dose standard maintained at a national standards laboratory. In this way, the dosimeter colour change is a direct measure of dose, and when calibrated can be used to determine dose within quantifiable uncertainty limits.

The relevant total uncertainty (σ_{total}) can be written as:

$$\sigma_{total} = (\sigma_{cal}^2 + \sigma_{map}^2 + \sigma_{rep}^2 + \sigma_{mach}^2)^{1/2}$$

where:
σ_{cal} = calibration uncertainty
σ_{map} = dose mapping uncertainty
σ_{rep} = dosimeter reproducibility
σ_{mach} = machine variability

Dose is the key processing parameter in food irradiation, and dosimetry is used to:

- Establish the facilities operating parameters necessary to deliver a given radiation dose (*e.g.*, conveyor speed or other parameters that determine the duration of exposure);
- Monitor the routine operation of the irradiation facility, in order to ensure that the irradiation process is under control and the correct irradiation dose is being received by a specific product.

During routine processing in a facility, a food receives a broad distribution of dose. The minimum dose ($\overline{D}$ min) received by the food must be sufficient to ensure that the intended purpose can be achieved (*e.g.*, inactivation of pathogenic food borne micro-organisms) and the maximum dose ($\overline{D}$ max) received must be less than that which would adversely affect the food, or be within a legal limit (*e.g.*, Directive 1999/2/EC requires the dose uniformity ratio of $\overline{D}$ max/ $\overline{D}$ min to be equal to or less than 3.0).

2.3.2 Measuring and monitoring the maximum dose ($\overline{D}$ max) and minimum dose ($\overline{D}$ min)

Dose mapping to determine the distribution of dose throughout a specific food product is a routine procedure used to determine the magnitude of, and location where, the maximum dose is received and the magnitude of, and location where, the minimum dose is received and to characterize how the dose is distributed. This procedure involves placing a sufficient number of dosimeters throughout a number

of test loadings of the food product, and irradiating it in the same manner as it is intended to treat that specific food product during the routine processing operation.

Often the location of the maximum dose and the location of the minimum dose are not accessible during routine processing (for example they may be located within the food itself) and $\overline{D}$ max and $\overline{D}$ min are monitored by using a dosimeter placed in a reference position that is easily accessible. During dose mapping, the mathematical relationship between the dose recorded by the dosimeter in the reference location and the maximum and minimum dose received by the food can be established. Therefore, when a food item is irradiated routinely, a dosimeter in a reference location can be used to monitor the minimum dose and the maximum dose received by each load of food. The irradiation facility can therefore show that no part of the treated food, received a dose below the minimum required to achieve the desired purpose and can also state the maximum dose delivered to the food (*e.g.*, to prove that the ratio of $\overline{D}$ max / $\overline{D}$ min was less than or equal to 3.0).

One key difference between gamma ray (or X-ray) and E-Beam irradiation is the dose-depth distribution, the depth of the maximum dose within the irradiated material and dose rate. With gamma rays (or X-rays), in a commercial irradiation facility, the maximum dose is effectively at the surface of the material (food) where the radiation is incident. However, with E-beam facilities the maximum dose is located beneath the surface where the E-beam is incident (see Annex).

2.3.3 Overall average dose ($\overline{D}$)

The overall average dose was the concept used to express acceptable radiation doses in the SCF opinion (SCF, 1986) and is defined mathematically in Directive 1999/2/EC. It is the average of the dose received by every spatial element of the food. This quantity cannot be measured directly, and can only be calculated approximately. The overall average dose is a concept applied only to food irradiation. The overall average dose was once used in international food irradiation standards but the concept was not found to be helpful to regulators nor operators of irradiation facilities, and the overall average dose is no longer used in international food irradiation standards such as the 2003 revised Codex General Standard (Codex Alimentarius, 1983) its associated Codex Code of Practice (Codex Alimentarius, 1979) and the International Standards on Phytosanitary Measures (FAO, 2003 and 2009).

The overall average dose has been defined by the following integral over the total volume of the goods:

$$\overline{D} = \frac{1}{M} \int p(x,y,z) d(x,y,z) \text{ dV}$$

where:

M = total mass of the treated sample
p = local density at the point (x, y, z)
d = local absorbed dose at the point (x, y, z)
dV = dx dy dz, the infinitesimal volume element which in real cases is represented by the volume fractions

The overall average dose can be calculated for homogenous products or for bulk goods of apparent homogenous density. In these situations and if the shape of the absorbed dose distribution curve through the product is well determined and the positions of minimum dose and maximum dose are known, the mean value of the average values of the minimum dose ($\overline{D}$ min) and the average values of the maximum dose ($\overline{D}$ max) can be a good estimate of the overall average dose:

$$\text{Overall Average Dose} \cong \overline{D} \approx \tfrac{1}{2} \left(\overline{D}_{max} + \overline{D}_{min} \right)$$

For example, Directive 1999/3/EC[9] permits dried aromatic herbs spices and vegetable seasonings to be irradiated to a maximum limit which is specified as an overall average dose of 10 kGy.
Therefore the maximum dose allowed is 15 kGy provided that the minimum dose received by the food is 5 kGy. This meets the dose uniformity ratio requirement of Directive 1999/2/EC, because the overall average dose is (15+5)/2 = 10 kGy and the dose uniformity ratio is ($\overline{D}$ max/ $\overline{D}$ min) is 15/5 = 3.0.

2.3.4 Summary of Maximum Dose, Minimum Dose and Overall Average Dose

When food is irradiated there are three values of dose that can be stated, the maximum dose, the minimum dose and the overall average dose. The maximum dose and minimum dose can be measured directly. The minimum dose is the key process parameter as the minimum dose received by the food must be greater than the dose required to ensure efficacy of the treatment. The maximum dose is also an important process parameter as the maximum dose received by the food must be less than any dose that would render the food unacceptable. Food irradiation facilities are capable of operating with a dose uniformity ratio ($\overline{D}$ max/ $\overline{D}$ min) less than 3.0.

In contrast, the overall average dose is not a processing parameter but is a concept once used in international standards, and still found in legislation (such as Directive 1999/2/EC). However, the overall average dose cannot be measured directly but can only be estimated. This is difficult for non-uniform foods that have variable densities and spatial geometries (*e.g.*, whole chicken carcasses). It should be noted that the concept of overall average dose is peculiar to food irradiation and is not used in any other area of irradiation technology (*e.g.* sterilization of medical devices and pharmaceuticals).

2.3.5 SCF opinions and dose limits

Where previous SCF opinions have considered dose limits for food irradiation it is not always clear if the opinion is expressed in terms of overall average dose or maximum dose. For example the SCF Opinion (SCF, 1992) in relation to the irradiation of Camembert cheese states "*the treatment of Camembert cheeses manufactured from raw milk with gamma-radiation at doses up to 2.5 kGy was acceptable from a health point of view*" but it is not clear if this 2.5 kGy dose is a limitation on maximum dose or overall average dose.
The same holds true for the SCF opinion of 2003 where it was concluded that "*On the basis of the information presently supplied to it, the Committee is still of the opinion that it is appropriate to specify a maximum dose for the treatment of certain food products by ionising radiation and that irradiated foodstuffs should continue to be evaluated individually taking into account the technological need and their safety*."

The Codex General Standard on irradiated food was revised in 2003 (CAC, 2003a) and states that "*For the irradiation of any food, the minimum absorbed dose should be sufficient to achieve the technological purpose and the maximum absorbed dose should be less than that which would compromise consumer safety, wholesomeness or would adversely affect structural integrity, functional properties, or sensory attributes. The maximum absorbed dose delivered to a food should not exceed 10 kGy, except when necessary to achieve a legitimate technological purpose*".
The revised Codex Standard no longer uses the concept of overall average dose but instead uses the processing parameters of minimum dose and maximum dose. In addition, it states that food can be

[9] Directive 1999/3/EC of the European Parliament and of the Council of 22 February 1999 on the establishment of a Community list of foods and food ingredients treated with ionising radiation. OJ of the European Communities L 66, 13.3.22 February 1999, p 1-2.

irradiated to doses above 10 kGy (this replaced a limit on dose in the previous Codex standard which stated that food should not be irradiated above an overall average dose of 10 kGy).

2.4. Use of irradiation in the European Union and third countries

2.4.1 European Union

In the European Union (EU) food irradiation is regulated by Directive 1999/2/EC and Directive 1999/3/EC. The latter Directive is the initial, and not yet completed, positive list of foods authorised for radiation treatment in the whole EU (contains so far only "dried aromatic herbs, spices and vegetable seasonings"). Until the completion of this initial positive list, Member States may maintain national authorisations for the irradiation of foods if these authorisations were given before the entry into force of the Directive and if the SCF gave a favourable opinion on the radiation treatment of this particular foodstuff. The list of national authorisations is published by the European Commission[10]. Member States can also maintain restrictions or bans on food irradiation until a final list of foods authorisation is established.

There are 23 approved food irradiation facilities in 12 Member States (Belgium, Bulgaria, Czech Republic, Germany, Spain, France, Hungary, Italy, The Netherlands, Poland, Romania and the United Kingdom). Approvals are granted by the competent authorities in Member States, in accordance with the procedure established by Directive 1999/2/EC.

Each year, Member States have to inform the European Commission about the amounts of food irradiated in the facilities on their territory. In addition they have to report the checks on food products placed on sale and the results of testing. The European Commission publishes these annual data. According to the last available report of 2007 (EC, 2009), 8,154 ton of food were irradiated in 16 of the approved irradiation facilities in eight Member States. The foodstuffs irradiated include: dried aromatic herbs, spices and vegetable seasonings, fresh and dried vegetables, dried fruits, various dehydrated products, starch, poultry meat, other types of meat, fish and shellfish, frog legs and frog parts, shrimps, egg white, eggs powder, dehydrated blood, gum arabic. These quantities and food categories include both foodstuffs placed on the EU market and foodstuffs exported to third countries.

Previous Commission annual reports relating to years from 2000 to 2006 were published in the EU official journal and are publicly available at the European Commission's website (EC, 2002; 2004; 2006a and b; 2007; 2008); a minimum of around 14,300 ton (in 2004) to a maximum of around 19,700 ton (in 2002) of food irradiated was reported in the EU.

In the EU, all irradiated food or food ingredients, have to be labelled as "irradiated" or "treated with ionizing radiation". This labelling is required even if an irradiated ingredient is present in trace amounts in a non-irradiated compound food. Analytical methods are available to detect irradiated food and control the compliance with labelling requests (see section 4).

2.4.2 Third countries

Food irradiation is approved for use in over 60 countries for various applications and purposes in a wide variety of foodstuffs; however, its use as a post-harvest phytosanitary (quarantine) treatment is still limited. Examples of countries with legislation allowing phytosanitary uses of irradiation include Argentina, Australia, Bangladesh, Brazil, China, India, Israel, Mexico, Philippines, Russia, Thailand, Turkey, Ukraine, the United States of America (USA), and Vietnam.

[10] OJ C 283/02, 24.11.2009, p.5.

As noted, irradiation may be used as a post-harvest phytosanitary treatment, especially for quarantine purposes. For example, the United States Food and Drug Administration (US FDA) recently approved in August 2008 the use of irradiation to control food-borne pathogens in iceberg lettuce and spinach at a maximum level of 4 kGy, in addition to existing USA approvals for a variety of products, including herbs and spices, grains, poultry, ground beef, and seafood (US FDA, CFR21[11]).

Kume *et al.et al.* (2009) published a study outlining the state of play of the use of food irradiation in the world in 2005, based on published data, a questionnaire survey and direct visits carried out in several countries throughout the world. They reported that the total volume of food irradiated worldwide in 2005 was 405,000 ton and classified the foods irradiated per food category or purpose and per geographic area, as briefly summarised in Table 2 below. China was the leading country in the use of food irradiation (146,000 ton) and three countries including USA (92,000 ton) and Ukraine (70,000 ton) made up three quarters of the total amount of food irradiated in the world in 2005. The authors reported that the main technology used is Co-60 irradiation, and that the use of E-beam and X-rays has recently increased.

Table 2. Volumes (ton) of food irradiated worldwide in 2005 (source Kume *et al.*, 2009).

Purpose	American region	European region	Asian and Oceania region	Africa and other region	*Total*
Disinfection of spices and dry vegetables	101,400	3,649	62,912	17,725	*185,686*
Disinfestation of grains and fruits	7,000	11	4,582	70,000	*81,593*
Disinfection of meat and seafood	8,000	9,263	15,208		*32,471*
Sprout inhibition of root crops and bulbs			88,196		*88,196*
Other food items (health foods, mushroom, honey *etc.*)		2,137	12,411	2,310	*16,858*
Total	*116,400*	*15,060*	*183,309*	*90,035*	*404,804*

Currently, the International Atomic Energy Agency (IAEA) is responsible for updating and maintaining various irradiation databases[12] as resources for researchers, government officials and the general public, including:

- *Food Irradiation Clearances Database*: a database on country approvals of irradiated foods for human consumption. The information includes country name, class of food, specific food product, objective of irradiation, date of approval and recommended dose limit.
- *Food Irradiation Facilities Database*: a database on facilities for the irradiation of foods intended for human consumption. The information includes country name, facility contact details, type of irradiator and energy source.

[11] US FDA Code of Federal Regulations 21CFR179.26
[12] See IAEA Nucleus at http://nucleus.iaea.org/NUCLEUS/nucleus/Content/index.jsp

Commission Decision 2002/840/EC[13,14] lays down a list of irradiation facilities located in third countries which are approved for irradiation of food to be imported in the EU. Ten facilities are included in that list, located in South Africa, Turkey, Switzerland, India, and Thailand.

3 Effects of ionising radiation on food

In general, the extent of chemical reactions induced by irradiation in food components depends on many variables. The most important are the irradiation treatment conditions like the absorbed dose, dose rate and facility type, presence or absence of oxygen and temperature. The composition of food and its physical state (frozen or fresh, solid, liquid or powder) also influence the extent of the reactions induced by the radiation treatment and the nature of the formed products. Chemical reactions and the products generated from major food components like fat, proteins, carbohydrates and vitamins are described in section 3.2. The nutritional value of irradiated foods is beyond the scope of this Opinion and outside the remit of the CEF Panel.

3.1 Generation of radiolytic products

Chemical changes can occur via primary radiolysis effects that result because of direct absorption of energy by the irradiated food, and also by secondary indirect effects. Here the high reactivity of the free radicals and excited molecular ions produced form very reactive intermediates. These can undergo a variety of reactions leading to stable chemical products, often referred to as radiolytic products.

Consequently, as ionising radiation passes through food it creates a trail of chemical transformations by primary and secondary radiolysis effects. It is instructive when considering the consequences with respect to any effect on the chemical composition of the food, to get some feel for the chemical yields involved. The yield will be food-specific and so a simple case is used here for illustration: the gamma-irradiation of aerated water. The initial products are an electron and a radical cation of water (Equation 1). In this simplified depiction the state of solvation by water molecules is not shown. The radical cation disassociates quickly into a proton and a hydroxyl radical (Equation 2).

Equation (1): $H_2O \rightarrow H_2O^{+\cdot} + e^-$

Equation (2): $H_2O^{+\cdot} \rightarrow H^+ + HO^{\cdot}$

The chemical yield of both intermediate (reactive) and final (stable) radiolytic products is often expressed using a convenient unit of the G-value. In aerated water the electron is scavenged and the G-value for the hydroxyl radical is 2.8. This means that 2.8 hydroxyl radicals are formed for each 100 eV (electron volts) of absorbed dose. Since 1 Gy (gray) = 6.24×10^{18} eV/dm^3 and taking account the Avogadro number, in aerated water with a G-value of 2.8 the yield of hydroxyl radical is 2.9×10^{-4} moles per dm^3 for each kGy of absorbed dose.

Placing this in a food context, assuming a food density of 1 kg/dm^3 and for a transformation product of, for example, molecular weight equal to 200 Dalton (Da), a 2.8 G-value would equate to 580 mg/kg of chemical product potentially formed in the food (or chemical loss by reaction) for each kGy of absorbed dose. Clearly when foods are irradiated in air the G-value will be different and there will be a multitude of transformation pathways available, so no single product will approach such high concentrations. On the other hand, if the ionizing radiation initiates a chain reaction (*e.g.*, autoxidation) then the chemical yield of the primary irradiation product (leading in this case to lipid

[13] Commission Decision of 23 October 2002 adopting the list of approved facilities in third countries for the irradiation of foods. OJ of the European Commission L 287, 25/10/2002, pp. 40 – 41.

[14] Commission Decision of 22 March 2010 amending Decision 2002/840/EC of 23 October 2002 as regards the list of approved facilities in third countries for the irradiation of foods. OJ of the European Commission L 75, 23/03/2010, pp. 33-34.

peroxyl radicals) can be multiplied by the chain-length. So the example does illustrate quantitatively the damaging potential of the typical doses used.

The formation of several chemical substances has been reported in the scientific literature as a result of radiation treatment of food. They belong to many chemical classes such as hydrocarbons, furans, alkylcyclobutanones, cholesterol oxides and aldehydes. Most of these substances are also formed in food that has been subjected to other processing treatments and are thus not exclusively formed by irradiation. However, the quantities in which they occur in irradiated food may vary considerably from those found in food subjected to other processes such as heat treatments. Until recently, only one class of chemical substances, the 2-alkycyclobutanones, were reported not to occur in non-irradiated food thereby indicating that they may be unique radiolytic products. In 2008, Variyar and co-authors (Variyar *et al.*, 2008) reported that they had detected 2-dodecylcyclobutanone (2-dDCB) and 2-tetradecylcyclobutanone (2-tDCB) in commercial non-irradiated and in fresh cashew nut samples, as well as 2-decylcyclobutanone (2-DCB) and 2-dDCB in non-irradiated nutmeg samples. The presence of 2-tetradecenylcyclobutanone (2-tDeCB) was also observed in both commercial and irradiated cashew nuts (Variyar *et al.*, 2008). As no further evidence of the natural occurrence of 2-alkylcyclobutanones (2-ACBs) has yet been reported, it would be pertinent to treat these findings with some caution until the results are validated by further experimental work.

3.2 Effects on food constituents

3.2.1 Proteins

The chemical reactions produced by the irradiation of proteins depend on several parameters like the protein structure (globular, fibrous), state (native or denatured), physical status (in solution, solid, frozen), amino acid composition, the presence of other substances in the food and, of course, the irradiation treatment. Major changes consist of dissociation, aggregation, cross-linking and oxidation. For instance gamma irradiation of hazelnuts at 10 kGy induced aggregation and denaturation of proteins resulting in moderate effects on the protein structure (Dogan *et al.*, 2007). The protein profile of black truffles treated with gamma rays at 2 kGy was characterised by a more rapid hydrolysis for certain proteins with 40 and 37 kDa and the generation of a 20 kDa polypeptide (Nazzaro *et al.*, 2007). Any structural change may affect its potential allergenicity as described in section 7. An important fact is that monomeric enzymes causing autolysis/hydrolysis can not be inactivated by irradiation, needing other treatments (*i.e.*, heating) to be inactivated (Delincée, 1983a). A study performed with four enzymes, these being microbial α-amylase, fungal α-amylase, glucoamylase and pectinase, showed a decrease in the respective enzyme activities with the absorbed dose range 1 to 20 kGy, when the enzymes were irradiated with either gamma rays or E-Beam irradiation. At 20 kGy, the reported decrease in activity was in the range 20% to 50%, with pectinase being the most sensitive enzyme to irradiation (Duliu *et al.*, 2004).

The main low molecular radiolytic products resulting from peptide irradiation consist of compounds such as ammonia, keto acids, amide-like products and diamino acids (Delincée, 1983a).

Radiation-induced amino acid modifications have been well documented. Aromatic and sulphur containing amino acids are most susceptible to irradiation. This is the case for the generation of three tyrosine isomers (para-, meta- and ortho-tyrosine) after ionizing radiation of phenylalanine (Hein *et al.*, 2000). α,α′-diamino acids have been identified after irradiation of amino acids like cysteine phenylalanine and glycine (Delincée, 1983a).

3.2.2 Lipids

The irradiation of lipids produces several chemical reactions the intensity of which depends on multiple factors such as lipid concentration, physical status (liquid or solid), unsaturation profile

(based on the composition in saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA)), presence of antioxidants, environmental conditions (light, heat, oxygen, moisture, pH), the irradiation treatment, type of storage (vacuum, modified atmosphere, *etc.*) and storage conditions (time, temperature, light, *etc.*) (Delincée, 1983b).

Irradiation has been shown to accelerate lipid oxidation (O'Bryan *et al.*, 2008; Stewart, 2009a). This effect is more relevant in foods with larger fat content and high unsaturated fatty acids content due to free radicals formed during irradiation. This effect may be minimised by using low temperatures and reducing the presence of oxygen (Stefanova *et al.*, 2010). The use of antioxidants is another alternative to retard lipid oxidation. A study by Nam and Ahn (2003) showed that antioxidant combinations using sesamol, gallate, and α-tocopherol effectively reduced lipid oxidation and off-odour volatiles, especially sulfur-containing volatiles, in pork patties treated with electron beam irradiation up to 4.5 kGy. However, it has been reported that antioxidative effects may be decreased in certain cases like the generation of volatile sulphur compounds that have a very low odour threshold (Fan, 2007). This work also indicated different mechanisms for such irradiation-induced effects. The type of packaging was found to have a greater impact than the antioxidant treatment (consisting of sesamol and α-tocopherol or gallate and α-tocopherol) to reduce the off-flavour caused by sulphur-compounds produced in E-beam irradiated (up to 3 kGy) turkey meat (Lee *et al.*, 2003). The use of natural antioxidants like oregano and rosemary extracts in beef burger subjected to E-beam irradiation with doses up to 7 kGy and stored frozen stored for up to 90 days, showed a great capacity to reduce lipid oxidation (da Trindade *et al.*, 2009).

2-Alkylcyclobutanones and certain hydrocarbons are generated from fatty acids present in the fat of a food being irradiated. These compounds are described in sections 4.3.1 and 4.3.2.

Cholesterol oxides also called “oxysterols” or “cholesterol oxidations products”, are produced by the oxidation of cholesterol, and are a group of sterols with a similar structure to cholesterol but containing an additional hydroxy, ketone or epoxide group on the sterol nucleous and/or a hydroxy group on the side chain of their molecules (Tai *et al.*, 1999, Savage *et al.*, 2002). There is no evidence in the literature of increased formation of cholesterol oxides in irradiated foods. The levels of cholesterol oxides in turkey meat, gamma irradiated at 3 kGy, were very similar to those in the untreated control meat: 3.06 versus 2.04 µg/g for 7α-hydroxycholesterol, 2.02 versus 2.12 µg/g for 7β-hydroxycholesterol, 2.70 versus 2.50 µg/g for 7-ketocholesterol and 2.28 versus 2.30 µg/g for cholesterol-5α, 6α-epoxide (Farkas *et al.*, 2009). Similar amounts of cholesterol oxides were also reported by other authors (Nam *et al.*, 2001) in non irradiated meat patties under vacuum, before storage, when compared to same samples irradiated at 4.5 kGy (see Table 3).

Table 3. Content of cholesterol oxidation products (COPs), expressed as µg/g lipid, in meat patties made from turkey leg, beef loin and pork loin in vacuum packaging at day 0, either non irradiated or irradiated at 4.5 kGy using E-beams (Adapted from Nam *et al.*, 2001).

COPs	Turkey		Beef		Pork	
	Non-irr	Irrad	Non-irr	Irrad	Non-irr	Irrad
7α- & 7β-hydroxy-cholesterol	31.2	34.3	7.8	5.6	10.6	9.1
β-Epoxide	0	0	0.8	1.1	3.0	1.9
α-Epoxide	0.6	3.9	5.6	3.6	8.4	3.0
20α-hydroxy-cholesterol	0	0	0.6	0.8	0	0
Cholestanetriol	0.6	0	0.6	0	0	0
7-Ketocholesterol	1.2	2.6	7.7	5.3	8.8	3.2
Total	33.5	36.8	23.0	16.3	30.8	17.2

The influence of packaging type during storage is very important as regards cholesterol oxidation. So, after electron beam irradiation at 2 kGy, the oxidation of cholesterol during storage at 4ºC for 7 days was found to be faster in aerobically packaged cooked chicken, 177.8 µg/g lipid, than in vacuum packaged cooked chicken that was 32.9 µg/g lipid (Lee *et al.*, 2001a). Similarly, a lower cholesterol oxidation products found in vacuum packaged meat in relation to aerobic packaged meat stored at 4ºC for 7 days were observed with patties irradiated at 4.5 kGy and made from turkey (134.7 vs 31.6 µg/g lipid for aerobic vs vacuum package, respectively), beef (69.9 vs 33.3 µg/g lipid) and pork (76.5 vs 24.6 µg/g lipid) (Nam *et al.*, 2001). The authors concluded that the type of packaging is more important than irradiation in terms of cholesterol oxidation (Nam *et al.*, 2001).

Phytosterols, which include sterols and stanols, are naturally present in cereals, nuts, seeds, fruits and vegetables. These compounds, which have a structure similar to cholesterol, can be oxidised by standard heating treatments and also by irradiation and produce oxyphytosterols even at low levels. In fact, electron beam irradiation of vegetable oils at up to 9.3 kGy was reported to have very low oxidising effect in β-sitosterol. The generated oxides were 7α-hydroxysitosterol, 7β-hydroxy-sitosterol, 5,6β-epoxy-sitosterol, 5,6α-epoxy-sitosterol, 5α,6β-dihydroxy-sitosterol and 7-keto-sitosterol. The total amount was lower than 24 µg/g in sunflower oil and lower than 4.5 µg/g, for olive, rapeseed and soybean oils (Zhang *et al.*, 2006). These values are similar to those reported for heating treatments. So, reported phytosterol oxidation products identified in heated oils were less than 17.6 µg/g of lipids (Johnsson and Dutta, 2006).

3.2.3 Carbohydrates

Mono- and polysaccharides are generally modified by ionizing radiations (Adam, 1983). The application of gamma-irradiation up to 6.2 kGy/h to starches from different foods like maize, wheat, rice or potato, induced the formation of aldehydes such as malonaldehyde, formaldehyde, and acetaldehyde, formic acid and hydrogen peroxide as main radiolytic products (Raffi *et al.*, 1981a; Raffi *et al.*, 1981b). The amounts of glyceraldehyde, dihydroxyacetone and 2-hydroxymalonaldehyde released after gamma irradiation of maize starch at different doses up to 50 kGy were reported to be within the ranges 0-8 µg/mL, 0-2.5 µg/mL and 0-9 µg/mL, respectively (Raffi *et al.*, 1981a; Raffi *et al.*, 1981b). In a more recent study (Fan, 2003), solutions of fructose, glucose, sucrose and starch at 90 mg/mL concentration were gamma irradiated at 3 kGy at 5ºC and the produced malonaldehyde, formaldehyde and acetaldehyde were quantified. The reported results showed the generation of about 0.45 µg/mL malonaldehyde, 1.1 µg/mL and 0.1 µg/mL acetaldehyde for the treated fructose solution. In the case of glucose solution, they were <0.1 µg/mL malonaldehyde, 0.15 µg/mL formaldehydeand traces of acetaldehyde while 0.3 µg/mL malonaldehyde, 0.8 µg/mL formaldehydeand traces of acetaldehyde were obtained for the treated sucrose solution. Finally, the treated starch solution

generated malonaldehydeat trace levels, 0.2 µg/mL formaldehydeand 0.2 µg/mL acetaldehyde (Fan, 2003). The degradation of starch polymers by gamma irradiation up to 10 kGy resulted in a change of some physicochemical properties like a decreased viscosity, increased water solubility and increased acidity (Kizil *et al.*, 2002; Sokhey and Chinnaswamy, 1993; Sokhey *et al.*, 1993).

The accumulation of aldehydes formed after irradiation of fruit juices may be lowered by reducing the presence of oxygen and using low temperature (Fan and Thayer, 2002).

Furans may be formed in ready-to-eat foods (RTE) containing ingredients like glucose, fructose or sucrose. A study made with aqueous solutions of such sugars at concentrations of 50 mg/mL and pH about 6.4, showed that gamma irradiation at 5 kGy was reported to produce 2- 7 ng/mL of furans, while 0- 3 ng/mL furans were produced by thermal treatment (autoclave for 25 min). The rate was substantially increased when pH decreased to 3 where up to 21 ng/mL furans were reported in the irradiated sugar solutions versus up to 25 ng/mL furans for the autoclave treatment (Fan, 2005b). Studies of furan formation in aqueous solutions of RTE ingredients like honey, corn syrup, sodium ascorbate and sodium erythorbate were also performed with gamma irradiation treatment up to 4.5 kGy (Fan and Sommers, 2006). Irradiation induced the formation of furans in such aqueous solutions but no radiation-induced furans were found in RTE meats containing such ingredients and exposed to 4.5 kGy radiation in nonfrozen state or 10 kGy in the frozen state (Fan and Sommers, 2006). A content of up to 9 ng/g of furans were reported in nonirradiated RTE foods (Fan and Sommers, 2006). Furthermore, the irradiation treatment was reported to reduce the furans formation in meats that contained high levels furans generated by thermal treatment (Fan and Mastovska, 2006) except for foods like orange juice, which are rich in carbohydrates and ascorbic acid, because the furan formation rate may exceed the reduction rate (Fan, 2005a and b).

3.2.4 Vitamins

Losses of vitamins may be caused by irradiation, similar to those reported for other processes involving heat treatment like pasteurisation or sterilisation. A comparison of vitamin content remaining in the food after high dose gamma-irradiation, E-beam irradiation or heat sterilisation treatments is shown in Table 4. As can be observed, vitamins losses are within similar ranges in all cases and thus, not much difference may be attributed to irradiation even though foods were treated at -25ºC, because vitamins are also sensitive to the heat treatments usually performed in the food industry. In some cases, there are some increased concentrations of several vitamins after irradiation treatment. This might be due to either an radiation-induced conversion of precursors to the active vitamin or even a better extraction efficiency from the food matrix (Diehl *et al.*, 1991; Diehl, 1992).

Water soluble vitamins have different sensitivities to irradiation. Thiamine is the most sensitive and significant losses can occur in meats, especially pork meat that contains high thiamine levels. Ionizing radiation of chicken meals with 1 kGy resulted in a 16% decrease in thiamine when compared to non-irradiated meals (Stewart, 2009a). In the case of folic acid an experiment undertaken with hamburgers enriched with up to 2.4 mg folic acid/100g revealed that folic acid levels decreased 20–30% following irradiation with a dose of 2 kGy;no additional decrease was observed at the higher dose of 4 kGy (Galan *et al.*, 2010). An ionising radiation of 2.5 kGy caused about 10% loss of total folates in spinach, green cabbage and Brussel sprouts (Müller and Diehl, 1996). Changes reported for total ascorbate in orange juice that was gamma irradiated up to 8.7 kGy revealed an approximate linear loss of 2.7% for each kGy increase (Fan *et al.*, 2002). Significant losses (10 to 34%) of total ascorbate have been reported for black pepper, cinnamon, nutmeg, oregano, and sage, as well as losses of carotenoids (about 40-60%) in cinnamon, oregano, parsley, rosemary, bird pepper, and sage after gamma irradiation at a dose of 10 kGy (Calucci *et al.*, 2003). Riboflavin, vitamin B_6, vitamin B_{12} and niacin have been reported as fairly stable to irradiation (Diehl, 1991); in absence of oxygen 10-15% losses of riboflavin were reported in wheatmeal, cornmeal and oatmeal after 10 kGy of gamma irradiation (Hanis *et al.*, 1988) and 13 and 16% losses of vitamin B_6 for cod and mackerel, respectively, after gamma irradiation at 1 kGy (Underdal *et al.*, 1976). No changes have been reported for niacin after

gamma irradiation with up to 10 kGy for mackerel and cod fillets as well as in wheat, maize mung beans and chick peas irradiated up to 5 kGy (Kilcast, 1994). No losses in vitamin B_{12} have been reported after 6.65 kGY gamma irradiation of pork chops (Fox *et al.*, 1989).

Fat soluble vitamins have different sensitivities to irradiation and have been shown to decrease in the following order: Vitamin E > β-carotene > vitamin A > vitamin D > vitamin K (Diehl, 1995).

Vitamin E is the most sensitive. About 44% less vitamin E was reported in oated rolls after E-beam irradiation with 1 kGy followed by storage for 6 months (Diehl, 1991). Losses of vitamin E, which is particularly sensitive to the presence of oxygen, might be minimised if food is irradiated in the absence of oxygen. Pork liver subjected to 5 kGy at 0ºC contained 4% less vitamin A than the non-irradiated control after one week and 13% less after 4 weeks of storage (Diehl, 1995). Losses of 2-7% for β-carotene have been reported in fresh milled wheat flour gamma irradiated at 1 kGy (Diehl *et al.*, 1991). Vitamin D has shown good resistance to irradiation as can be observed in Table 4 . It is important to take into account that losses are generally smaller when vitamins are irradiated in a food matrix rather than in pure solutions (Zegota, 1988). Furthermore, vitamin losses may be minimised if food irradiation takes place at freezing temperatures and in the absence of oxygen (Diehl, 1991; WHO, 1999).

Table 4. Vitamin content of frozen, thermally processed, gamma-irradiated and electron-irradiated enzyme-inactivated chicken meat[a] (reproduced with permission from WHO (1999).

	Process			
Vitamin	***Frozen control***	***Heat-sterilized***	***Gamma-irradiated (59 kGy at – 25ºC)***	***Electron-irradiated (59 kGy at – 25ºC)***
Thiamine hydrochloride (mg/kg)	2.31	1.53[b]	1.57[b]	1.98
Riboflavin (mg/kg)	4.32	4.60	4.46	4.90[c]
Pyridoxine (mg/kg)	7.26	7.62	5.32	6.70
Nicotinic acid (niacin) (mg/kg)	212.9	213.9	197.9	208.2
Pantothenic acid (mg/kg)	24.0	21.8	23. 5	24.9
Biotin (mg/kg)	0.093	0.097	0.098	0.103
Folic acid (mg/kg)	0.83	1.22	1.26	1.47[c]
Vitamin A (IU/kg)	2716	2340	2270	2270
Vitamin D (IU/kg)	375.1	342.8	354.0	466.1
Vitamin K (mg/kg)	1.29	1.01	0.81	0.85
Vitamin B12 (mg/kg)	0.008	0.016[c]	0.014[c]	0.009

[a] Vitamin concentrations are given on a dry weight basis. [b] Significantly lower than frozen control. [c] Significantly higher than frozen control. Adapted from (Thayer, 1990) with the permission of the publisher.
WHO gave permission but the original table was from Thayer (1990).

3.2.5 Inorganic salts

Inorganic anions are relatively unreactive towards primary radicals, except for nitrates which are reduced by solvated electrons to nitrites. This is considered to be a rare event in frozen muscle foods because of the competition for electrons by the other matrix constituents (SCF, 2003).

Gamma irradiation could be helpful to increase the capacity of ascorbic acid to reduce the nitrite and thus reduce the possible formation of N-nitrosamines, especially nitrosodimethylamine and nitrosopyrrolidine, in meat products (Ahn *et al.*, 2004) and then, minimise the risk for nitrosamine formation. So, the generation of volatile N-nitrosamines in pork sausage were reported to decrease by gamma irradiation up to 20 kGy during storage for 4 weeks under refrigeration (Ahn *et al.*, 2002). Similar nitrosamines reductions were reported in cooked pork sausages treated with 5 kGy and stored for up to 4 weeks under refrigeration (Jo *et al.*, 2003).

3.3 Effects on other food constituents

Irradiation can also affect other food constituents. The phenolic content and antioxidant capacity of fresh-cut vegetables like Romaine and Iceberg lettuce and endive were reported to be increased, more in the midrib than in the non-midrib leaf tissues, by gamma irradiation up to 2 kGy. However, the increase in phenolics makes such vegetables prone to undesirable browning reactions (Fan, 2005b). The flavonoids (+)-catechin, (-)-epicatechin and glycosides from kaempferol and quercetin, as well as four phenolic acids (gallic acid, p-coumaric acid, caffeic acid and 4-hydroxybenzoic acid) were studied in strawberries gamma irradiated up to 6 kGy. It was found that all flavonoids decreased in concentration except for quercetin-3-glucoside which remained unaffected. The phenolic acids remained largely unaffected except for 4-hydroxybenzoic acid which increased in concentration (Breitfellner *et al.*, 2003).
Irradiation may induce cis-trans isomerization and thus, some trans-fatty acids may appear in irradiated foods. The amount of trans fatty acids was reported to increase with the increase of gamma irradiation dose (from 0 to 8 kGy) in ground beef in comparison to the control (Brito *et al.*, 2002). However, these levels did not increase after storage up to 90 days at -10ºC. Nearly no cis–trans-isomerization of fatty acids could be detected in barley grains treated with gamma irradiation up to 10 kGy. When a higher dose was used (50 kGy) the trans-fatty acid concentration was similar to that found in other natural products like milk fat (Geissler *et al.*, 2003).

Some effects on anti-nutrients have also been reported after irradiation treatment although the radiation doses used were much higher than those recommended as acceptable by SCF (2003). Partial inactivation of trypsin inhibitor activity (TIA) and haemaglutinin was achieved in proportion to the gamma radiation dose; for instance, 41.8% reduction in TIA at 5 kGy to 72.5% reduction at 60 kGy (El-Niely, 2007). The content of tannins and phytate in uncooked and cooked common beans (*Phaseolus vulgaris*) decreased when the gamma irradiation dose increased up to 10 kGy (Brigide and Canniatti-Brazaca, 2006). When food containing DNA is irradiated, modification of these large molecules occurs including fragmentation (Delincée *et al.*, 2002). The hydroxyl radical generated radiolytically may react with the purine and pyrimidine bases in DNA but also the strand breaks or crosslinking between bases and proteins may result in relevant structural changes.

3.4 Effect on food additives

Certain additives may be affected by irradiation and contribute to a potential generation of harmful radiolytic products into the food. As an example, this appears to be the case of potassium benzoate that was used as antimicrobial at 0.1% content in RTE turkey ham treated with up to 2.0 kGy followed by up to 6 weeks of storage under refrigeration. The analysis of volatile compounds by gas chromatography-mass spectrometry (GC-MS) revealed the formation of benzene in the irradiated ham samples which can be explained by decarboxylation of the contained potassium benzoate (Zhu *et al.*,

2005). This is similar to the potential generation of benzene in acidic beverages like soft drinks containing benzoic acid and ascorbic acid (McNeal *et al.*, 1993, Gardner and Lawrence, 1993).

4 Detection methods for irradiated foods

Physical, chemical, biological and microbiological changes induced by radiation treatment may be analysed to differentiate between irradiated and non-irradiated foods. Studies on the detection of irradiated foods are recorded as far back as the 1960s but only in the 1990s did research programmes of the European Commission as well as the Food and Agricultural Organisation and the World Health Organisation (FAO/WHO) result in the coordination of the multiple international activities being undertaken. Within these work programmes, detection methods were developed and/or improved. The most promising methods were tested via large scale inter-laboratory studies for their reliability and suitability for use in official food control. Under the mandate of the European Commission ten validated methods were standardised by the European Committee for Standardisation (CEN) as European Standards (EN) (see Table 5). These standardised methods were later recognised internationally as Codex Alimentarius General Methods. Today they are widely used by official, as well as private laboratories, to check food products or their ingredients for radiation treatment.

Table 5. European standards for the detection of irradiated foodstuffs (from Stewart, 2009b)

EN1784:2003	**Foodstuffs – detection of irradiated food containing fat – gas chromatographic analysis of hydrocarbons**
	Validated with raw meat, Camembert, fresh avocado, papaya, mango
EN1785:2003	**Foodstuffs – detection of irradiated food containing fat – gas chromatographic/mass spectrometric analysis of 2-alkylcyclobutanones**
	Validated with raw chicken, pork, liquid whole egg, salmon, Camembert
EN1786:1996	**Foodstuffs – detection of irradiated food containing bone – method by ESR spectroscopy**
	Validated with beef bones, trout bones, chicken bones – expected that method can be applied to all meat and fish species containing bone
EN1787:2000	**Foodstuffs – detection of irradiated food containing cellulose, method by ESR spectroscopy**
	Validated with pistachio nut shells, paprika powder, fresh strawberries
EN1788:2001	**Foodstuffs – detection of irradiated food from which silicate minerals can be isolated, method by thermoluminescence**
	Validated with herbs and spices as well as their mixtures, shellfish including shrimps and prawns, both fresh and dehydrated fruits and vegetables, potatoes
EN13708:2001	**Foodstuffs – detection of irradiated food containing crystalline sugar by ESR spectroscopy**
	Validated with dried figs, dried mangoes, dried papayas, raisins
EN13751:2002	**Detection of irradiated food using photostimulated luminescence**
	Validated with shellfish, herbs, spices, seasonings
EN13783:2001	**Detection of irradiated food using Direct Epifluorescent Filter Technique/Aerobic Plate Count (DEFT/APC) - Screening method**
	Validated with herbs and spices
EN13784:2001	**DNA Comet assay for the detection of irradiated foodstuffs - Screening method**
	Validated with chicken bone marrow, chicken muscle, pork muscle, almonds, figs, lentils, linseed, rosé pepper, sesame seeds, soya beans, sunflower seeds
EN14569:2004	**Microbiological screening for irradiated foodstuffs – Screening method**
	Validated for chilled or frozen chicken fillets (boneless) with or without skin

4.1 Biological and microbiological screening methods

It should be noted that some of the methods, referred to as 'screening methods' are used only to indicate a processing treatment, possibly treatment with ionising radiation. These methods include measurement of the ratio of living to dead micro-organisms (EN 13783 DEFT/APC; EN 14569 LAL/GNB) or DNA strand breakage (EN 13784 DNA Comet Assay).

The Direct Epifluorescent Filter Technique/Aerobic Plate Count (DEFT/APC) method has been used on spices (Choi *et al.*, 2004a; Choi *et al.*, 2004b; Oh *et al.*, 2003), whereas the DNA Comet assay has been applied to a wide range of foods, such as papaya, melon, watermelon and kiwi (Jo and Kwon, 2006; Marin-Huachaca *et al.*, 2004), pork, poultry, beef and other types of meat (Araujo *et al.*, 2004; Khan *et al.*, 2003; Marin-Huachaca *et al.*, 2005; Villavicencio *et al.*, 2004a; Villavicencio *et al.*, 2004b), quail meat and seafood (Erel *et al.*, 2009; Khan *et al.*, 2003), and cereals and nuts (Khan *et al.*, 2005a; Khan *et al.*, 2005b).

Positive results from these methods should be confirmed using radiation-specific reference methods as referred to below.

4.2 Physical reference methods

Three of the EN methods use the technique of electron spin resonance (ESR) spectroscopy to detect free radicals formed by ionizing radiation in solid and dry components of food, such as in the hydroxyapatite of meat or fish bones (EN 1786), in the cellulose of the shells of nuts, of spices or seeds (EN 1787), and in the crystalline sugars of foods such as dried figs, dried mangoes, dried papayas or raisins (EN 13708). Other physical methods used for the detection of radiation-specific changes use luminescence techniques. Thermoluminescence analysis is applied to those foodstuffs from which silicate minerals can be isolated, e.g. herbs and spices, shellfish or fruit and vegetables. When exposed to ionizing radiation silicate minerals store energy in charge carriers trapped at structural, interstitial or impurity sites. The stored energy is released by controlled heating of isolated silicate minerals with light being emitted, the intensity of the emitted light being measured as a function of temperature resulting in a so-called 'glow curve' (EN 1788). Photostimulated luminescence uses optical stimulation of minerals to release stored energy (EN 13751). The same spectra can be obtained from whole herbs and spices and other foods using PSL and as the measurements do not destroy the sample, whole samples, or other mixtures or organic and inorganic material can be measured repeatedly.

ESR has been used to identify radiation treatment of various foods such as lamb meat (Chawla and Thomas, 2004), other types of meat, fish and shellfish (Miyahara *et al.*, 2004; Sin *et al.*, 2005), mechanically recovered poultry meat (Marchioni *et al.*, 2005a and b), kiwi (Jo and Kwon, 2006), oats (Korkmaz and Polat, 2004), melon, pumkin and sunflower seeds (Sin *et al.*, 2006), and cashew nuts (Bhaskar *et al.*, 2008).

Further validation studies of the thermoluminescence - and photostimulated luminescence -methods on spices, herbs, seasoning, fruit, vegetables and shellfish have been performed by Sanderson *et al.* (Sanderson *et al.*, 2003a, 2003b, 2003c). The thermoluminescence and/or photostimulated luminescence methods were used to identify radiation treatment of spices, tea, dried fruit, chestnuts and nuts (Bayram and Delincée, 2004; Bortolin *et al.*, 2006; Choi *et al.*, 2004a; Choi *et al.*, 2004b; Engin, 2004, 2007; Kitis *et al.*, 2005; Leth *et al.*, 2006; Mangiacotti *et al.*, 2009; Marchioni *et al.*, 2005b) and herbal supplements and herbal extracts (Boniglia *et al.*, 2009; Bortolin *et al.*, 2009).

4.3 Chemical reference methods

Radiation specific chemical changes in foodstuffs can be analysed by two EN methods, EN1784 and EN1785. Both are based on the measurement of radiolytic products formed from lipids.

4.3.1 Hydrocarbons

EN1784 is based on the detection of certain radiolytic hydrocarbons. In the fatty acid moieties of triglycerides, chemical bonds may break during treatment with ionising radiation mainly in the α– and β–positions with respect to the carbonyl groups resulting in the formation of respective C_{n-1}- and $C_{n-2:1}$-hydrocarbons[15] (see table 6). Thus, the radiation-induced hydrocarbon pattern can be predicted based on the fatty acid composition of the respective food. Using gas chromatographic separation of the hydrocarbon fraction and its mass spectrometric detection a given food sample can be identified as irradiated if the full expected pattern of C_{n-1}- and $C_{n-2:1}$-hydrocarbons is detected although the hydrocarbons yielded are not radiation-specific by themselves.

Table 6. Radiation induced C_{n-1}- and $C_{n-2:1}$-hydrocarbons and 2-alkylcyclobutanones produced from the major fatty acids (FA) in food

Fatty acid	C_{n-1}-hydrocarbon	$C_{n-2:1}$-hydrocarbon	2-Alkylcyclobutanones
Palmitic acid (FA 16:0)	Pentadecane 15:0	Tetradecene 1-14:1	2-Dodecylcyclobutanone (2-dDCB)
Stearic acid (FA 18:0)	Heptadecane 17:0	Hexadecene 1-16:1	2-Tetradecylcyclobutanone (2-tDCB)
Oleic acid (FA 18:1)	Heptadecene 8-17:1	Hexadecadiene 1,7-16:2	2-Tetradecenylcyclobutanone (2-tDeCB)
Linoleic acid (FA 18:2)	Heptadecadiene 6,9-17:2	Hexadecatriene 1,7,10-16:3	2-Tetradeca-5',8'-dienylcyclobutanone (2-tDdeCB)

The amount of C_{n-1}- and $C_{n-2:1}$-hydrocarbons in irradiated chicken, pork, beef and ostrich meat is in the range of 0.05 to 0.9 µg/g fat/kGy (Chung *et al.*, 2004; Morehouse and Ku, 1993; Nawar *et al.*, 1990; Sjoberg *et al.*, 1990).

Radiation treatment could be proved via the detection of radiolytic hydrocarbons in a variety of fatty foods, like cashew nuts (Bhattacharjee *et al.*, 2003), soybeans (Hwang *et al.*, 2007), dried shrimp and beef, pork and chicken meat (Kim *et al.*, 2004) and dry-cured ham (Blanch *et al.*, 2009). Barba *et al.* (Barba *et al.*, 2009) used supercritical fluid extraction (SFE) for the isolation of radiolytic hydrocarbon from cheese prior to identification by GC-MS.

4.3.2 2-Alkylcyclobutanones

EN1785 is used to identify 2-alkylcyclobutanones (2-ACBs) in irradiated food containing fat. The formation of these compounds was first reported by Letellie and Nawar in 1972 (Letellie.Pr and Nawar, 1972) (who proposed that the 2-ACBs result from the cleavage of the acyl-oxygen bond in triglycerides and have the same number of carbon atoms as the parent fatty acid with the alkyl group located in ring position 2). Thus, if the fatty acid composition of the foodstuff is known, the 2-ACBs produced upon radiation treatment can be predicted to a certain degree. As noted in Table 5, from the

[15] C_{n-1}-hydrocarbon has one carbon atom less than the parent fatty acid; $C_{n-2:1}$-hydrocarbon has two carbon atoms less than the parent fatty acid and an additional double bond in position 1;

four major fatty acids in foodstuffs, palmitic, stearic, oleic and linoleic acid, the corresponding cyclobutanones are formed, namely 2-dodecylcyclobutanone (2-dDCB), 2-tetradecylcyclobutanone (2-tDCB), 2-tetradecenylcyclobutanone (2-tDeCB) and 2-tetradeca-5',8'-dienylcyclobutanone (2-tDdeCB) (Horvatovich *et al.*, 2005; Letellie.Pr and Nawar, 1972). Once extracted from the foodstuff, the compounds can be separated using gas chromatography and detected using a mass spectrometer (EN 1785).

A significant body of evidence exists to demonstrate the usefulness of 2-dDCB and 2-tDCB as markers for the detection of a range of irradiated foodstuffs (Blanch *et al.*, 2009; Lee *et al.*, 2008; Obana *et al.*, 2006, 2007; Sin *et al.*, 2006; Stewart *et al.*, 2000; Zanardi *et al.*, 2007). Recent studies by Tewfik and Tewfik (Tewfik and Tewfik, 2008a and b) have investigated if these compounds could be used to identify irradiate lipid-containing foods towards the end of their shelf-life. Results showed a linear decrease in the amounts of 2-dDCB and 2-tDCB in irradiated beef and chicken by 50% over the storage period of one year at -20°C. However, despite the considerable drop in the levels of both markers with time, it was still possible to detect both 2-dodecylcyclobutanone (2-dDCB) and 2-tDCB and thereby confirm that the stored samples had been irradiated. Earlier work by Obana *et al.* (2006) also demonstrated that they appear to be stable in the foodstuffs treated up to a temperature of 100°C but are destroyed above 200°C.

By modification of the standardized method EN1785 the presence of only 0.5% of irradiated (5 kGy) mechanically recovered meat in food preparations could be detected via the analysis of 2-dDCB (Marchioni *et al.*, 2002). Caja *et al.* (Caja *et al.*, 2008) used solid phase microextraction as a method for the detection of 2-dDCB in ground beef, while Tewfik (Tewfik, 2008a and b) validated the use of the direct solvent extraction method for the identification of 2-dDCB in irradiated chicken and whole liquid egg.

The amount of 2-dDCB formed by irradiation with 5 kGy was determined to be 0.2 µg/g of fresh meat. Other authors found 2-dDCB in commercial irradiated ground beef at levels of 0.03 - 0.05 µg/g (Gadgil *et al.*, 2002; Gadgil *et al.*, 2005), 0.1 µg/g lyophilized poultry meat per kGy after 28 days of storage at 4°C (Horvatovich *et al.*, 2005) and 0.04 to 0.6 µg/g of lipids per kGy in irradiated chicken meat and beef (AFFSA, 2007; FDA, 2005). It should be noted that as well as 2-dDCB and 2-tDCB being detected has also been identified in chicken and pork meat, in papaya and mango (Stewart *et al.*, 2001; Zanardi *et al.*, 2007).

Until recently, the 2-ACBs have not been detected in non-irradiated foods or microbiologically spoiled products with extensive experimental work indicating that they are not produced by cooking, by packaging in air, vacuum or carbon dioxide, or during storage (Stevenson, 1994).

Variyar *et al.* (2008) reported about the presence of 2-ACBs in non-irradiated cashew nuts and nutmeg. 2-dDCB was found both in samples of non-irradiated and irradiated cashew nuts (1kGy, gamma rays) and in nugmet (5kGy, gamma rays). In addition, 2-tDeCB and 2-tDCB were detected in cashew nuts and 2-dDCB in nutmeg. The concentrations reported for the non irradiated cashew nuts were 1 microg/g for 2-tDCB, 2.7 microg/g for 2-dDCB and 0.52 microg/g for 2-tDeCB.The authors employed various extractions and purification methods; GC-MS was used for detection and quantification. The extraction and purifications method seemed to have a major influence on the concentration and pattern of the detected ACBs. Both, extraction with supercritical fluid (SFE) and Soxhlet extraction followed by purification on silica gel column did not lead to the detection of the ACBs in non-irradiated samples. Only the combination of SFE with silica gel thin layer chromatography (TLC) proved to be successful (recovery rate 96%) for the extraction of the ACBs in non-irradiated food.

Experiments to determine the detection limit (LOD) for 2-dDCB were described, reporting a LOD of 6.09 pmol. Recovery experiments for 2-tDeCB were also described but related only to the potential loss during the TLC cleaning step For each of the three different extraction methods three samples were analysed in duplicate.

No further evidence of the natural existence of 2-alkylcyclobutnaones has been reported since publications of this paper by Variyar *et al.* (2008). Thus, as noted previously it would be pertinent to treat these findings with some caution until the results are validated by further experimental work.

5 Toxicological aspects of irradiated food

In 1999, the Joint FAO/IAEA/WHO Study Group (WHO, 1999) reviewed the scientific literature on *in vivo* toxicological studies for the evaluation of irradiated food and concluded that foods treated with a dose greater than 10 kGy can be considered chemically safe and nutritionally adequate (WHO, 1999). This report included 27 peer-reviewed publications that mostly report negative results but rule out five peer-reviewed publications (four on rodents and one on monkeys) due to experimental deficiencies. Previously, in 1986 the US FDA reviewed over 400 studies and stated that only five of the studies reviewed were considered to have been properly conducted, fully adequate by 1980s standards, and capable of standing alone to support the safety of irradiated foods (FDA, 1986). The Study Group included some of the trials disregarded by the US FDA in its own evaluation.

The conclusions of these studies assessed by the Study Group (WHO, 1999) have been outlined and were also discussed in the technical report of the Food Standards Agency Project A05009 (Fielding, 2008). The WHO Study Group concluded that food irradiated to any dose appropriate to achieve the intended technological objective is both safe to consume and nutritionally adequate and irradiated foods are deemed wholesome throughout the technologically useful dose range from below 10 kGy to envisioned doses above 10 kGy. However, the Fielding report emphasizes the deficiencies in the methodology of these studies dated before approximately 1990 (*e.g.*, method of statistical analysis, exact conditions under which the irradiation was performed).

In 2007, the French Food Safety Agency (AFSSA) indicated that the examination of recent scientific literature does not provide any new information which might call into question, particularly in toxicological terms, the conclusions of the evaluations conducted to date which have determined that food treated with ionising radiation is safe. However, based on the trials evaluated, there is insufficient data about the levels of radiolytic products ingested by the animals and humans (AFSSA, 2007).

5.1 Genotoxicity studies (*in vitro* and *in vivo*) of irradiated food

5.1.1 *In vitro* genotoxicity studies of irradiated food

Several *in vitro* studies were carried out on irradiated constituents of foods or foods themselves in the past. Overall these studies have been considered negative, as shown, for istance, in the review of Ashley *et al.* (2004). However, in order to test irradiated foods *in vitro* it is necessary to prepare natural juices or extracts of them, thereby giving rise to several limitations in these tests, such as incomplete water and alcohol extractions of radiolytic products, low sensitivity, altered macromolecular structures, interference with the test system etc.

Over the last decade a number of studies not covered by the review of Ashley *et al.* (2004) have been published. In general these studies are considered negative according to the authors, for example the study of Young-Beob and Sung-Kee (Young-Beob and Sung-Kee, 2000) investigating the *in vitro* genotoxicity of a water extract from gamma-irradiated (10 kGy) *Angelica gigas* (Danggui) using Salmonella reversion assay (Ames test) and micronucleus test in Chinese hamster ovary (CHO) cells. Negative results were also reported for *in vitro* studies on gamma irradiated (1.5-30 kGy) foods, such as medicinal herbs, juices, egg white, anchovy sauce, porridge, fermented cabbage, black truffles. Studies included the Ames test, the chromosome aberrations in mammalian cells, the micronucleus test and the SOS chromotest (Hong-Sun *et al.*, 2004; Hyun-Ja *et al.*, 2001; Il-Jun *et al.*, 2005; Sung-Kee *et al.*, 2001; Kim *et al.*, 2003; Yu et al*;* 2004; Yook *et al.*, 2004 and 2005; Kang et al*;* 2005; Nazzaro *et al.*, 2007).

5.1.2 *In vivo* genotoxicity studies of irradiated food

There are many *in vivo* studies available on irradiated food constituents or foods themselves (Ashley *et al.*, 2004). However, most studies were carried out in the 1970s and 1980s and would not meet current testing standards. In addition the sensitivity was rather low to detect effects of small quantities of specific radiolytic products such as the 2-ACBs formed in the irradiated foods given to animals. FDA reviewed in the past (up to 1991; FDA 1986, 1987 and 1991) almost 60 mutagenicity studies with a few incidental positive results which could not be further confirmed. In 2005, a further 15 mutagenicity studies (*in vitro/in vivo)* were reviewed by the FDA and confirmed previous negative results.

In 2005 (Yook *et al.*, 2005) published an OECD-compliant study on the genotoxicological safety of fermented vegetables pasteurised by gamma-irradiation. The potential clastogenic effects of fermented cabbage extracts treated with 20 kGy gamma irradiation were tested *in vivo* in male ICR mice. Four groups of 6 male ICR mice were exposed to a dose of 2000 mg/kg bw of the water and methanolic extracts from irradiated and non-irradiated cabbage. The test for *in vivo* clastogenic effects in mouse bone marrow cells gave negative results.

Overall, there is little new information as regards genotoxicity of irradiated foods *in vivo*. The one new study from Yook *et al.* (2005) adds to the previous negative findings.

5.2 Subchronic studies of irradiated food

The FAO/IAEA/WHO Study Group concluded on the basis of the subchronic toxicity studies performed in mammals and avian species that the few adverse effects found were linked with nutritional inadequacy and not unique to irradiation (WHO, 1999).

In a subchronic toxicity study 20 Sprague-Dawley rats (10 males and 10 females) were fed irradiated (5.0 kGy) thaumatin (proteinaceous sweetener) at increasing concentration (0.3%, 1% and 3% in feed, equivalent to 260-299, 788-1042 and 2502-2889 mg/kg bw/day for males and females respectively) continuously for 90 days. The rats did not reveal any adverse effects attributable to the treatment. There were no toxicologically significant effects on the survival rates, body weight, feeding, urinalysis, blood chemistry parameters, and ophthalmology. The full histopathological examination carried out (more than 38 tissues and organs) did not reveal any adverse effects attributable to the consumption of irradiated thaumatin (Hagiwara *et al.*, 2005).

The recent scientific literature reviewed does not provide any new information on subchronic toxicity studies performed in animals fed diets containing irradiated food.

5.3 Chronic/carcinogenicity studies of irradiated food

The FAO/IAEA/WHO Study Group (WHO, 1999) reviewed the studies on two year carcinogenicity bioassays and multi-generation reproductive toxicology performed in mammals. Additional carcinogenicity bioassays without reproductive components have been reported for rats and mice. No irradiation-related increase in tumours in any of the studies using high-dose irradiated food or diets or changes in reproductive function in the multi-generation reproduction phases of the combined carcinogenicity-reproduction studies were noted in rats or mice. Similarly, there was no evidence of pathological findings in male rhesus monkeys consuming peaches irradiated at high-dose (27.9 and 55.8 kGy) during two years (the female monkeys had a problem with palatability of the irradiated diet). In the chronic studies carried out in dogs (2-4 years duration) there was no evidence of pathological abnormalities associated with high-dose irradiated food, and was concluded there were no indications of pathological abnormalities in any of these chronic study conducted in this animal species.

In addition, in mice and dogs (irradiated feed at doses of 27.9 and 55.8 kGy), there was no evidence of testicular tumours as consequence of consuming irradiated food as opposed to thermally treated chicken (FDA, 1997).

Overall, it can be concluded that the food irradiation has not demonstrated any treatment-related effect in the animal species studied.

5.4 Reproduction and developmental toxicity studies of irradiated food

The research performed during many years has demonstrated that there is no increased risk of cancer or birth defects with long-term consumption of irradiated meat and poultry in feeding studies using different animal species (FDA, 1997). There are, in fact, a small number of studies that produced equivocal results being connected with the safety of irradiated foods. However, in depth review of those studies determined that they were deficient in experimental design, and used insufficient numbers of animals for statistical analysis (WHO, 1994).

The FAO/IAEA/WHO Study Group (WHO, 1999) reviewed the studies on reproduction and teratology carried out in mammals. In the first study performed on rats, there were no detectable differences with respect to growth, feed consumption, reproduction, haematology, urinary and organ histopathology parameters between animals feed with an irradiated diet (50 kGy) or autoclaved diet (15 minutes at 120ºC). In the second study performed on pigs and involving three generations, no differences exist between feed consumption, growth, and haematological and biochemical parameters along the three generations, and it was concluded that there were no treatment-related effects in the growth and reproduction of pigs fed irradiated or autoclaved feed for three generations. The pigs from the F1a generation were slaughtered and the authors concluded that there were no treatment-related effects in feed consumption, growth, mortality, haematology, biochemistry of blood or urine, organ weights, histopathology or tumour incidence in animals fed irradiated or autoclaved feed.

The FAO/IAEA/WHO study group (WHO, 1999) also reviewed a long-term toxicity study carried out in rats fed a composite feed diet, each irradiated to 55.8 kGy and a decreased weight gain in females of the F3 generation, although caution was urged in interpreting the results because of the small number of animals used (Read *et al.*, 1961) However, it was concluded that the variations in reproductive performance: did not indicate toxicity although again caution should be used when interpreting these results. A review of the evidence of lesions in various organ tissues representing over 3000 rats did not indicate any macroscopic or microscopic lesions that could be specifically attributed to the irradiated diet (Read *et al.*, 1959).

Finally, FAO/IAEA/WHO Study Group (WHO, 1999) reviewed several multi-generation reproduction studies conducted on rodents and dogs (irradiated feed at doses of 27.9 and 55.8 kGy). Overall, minor effects were noted in some cases (*i.e.* small decreases in body weight or body weight gain in the later generations of multi-generation studies), which appear to be related to nutrition and reduced palatability of the diet.

Overall, it was concluded that there were no toxicological indications that attributable to irradiation.

5.5 Cases of cats disease related to consumption of irradiated pet feed

Several authors reported a disease called leukoencephalomyelopathy (LEM) in specific pathogen free (SPF) cat colonies in Britain (Palmer and Cavanagh, 1995) and New Zealand (Hendricks *et al.*, 2001). The clinical symptoms are progressive hind limb ataxia which seems to be caused by wallerian-type degeneration of parts of the central nervous system (spinal cord and various regions in the brain). Its aetiology has not been determined nor have the authors reported whether cats had been fed with irradiated diet.

Cases of leukoencephalomyelopathy were also reported in a colony of SPF cats in an Irish laboratory (Cassidy *et al.,* 2007) and with Australian domestic cats (Child *et al.*, 2009). Both authors associated the development of the disease to feeding with gamma-irradiated diet. The clinical sign, consisting of progressive hind limb ataxia, could be reproduced experimentally in cats (Caulfield *et al.*, 2009; Duncan *et al.*, 2009).
Spontaneous occurrence of a clinico-pathological similar syndrome, of undetermined aetiology, has also been described in other felines, like cheetahs, leopards and lions (Palmer *et al.*, 2001; Maratea *et al.*, 2006; Cassidy et al, 2007).

5.5.1 Leukoencephalomyelopathy case linked to irradiated feed ingestion

In the first case linking LEM to irradiated feed ingestion (Cassidy et al, 2007), three outbreaks of the LEM syndrome occurring between 1998 and 2001 in Ireland are reported in a specific pathogen free (SPF) cat colony in a research laboratory. A total of 190 out of 540 domestic short-hair cats developed hind limb ataxia and proprioceptive defects during a period of four years. Both male and female animals were affected, and the age ranged from 3 months to 3 years. The colony was established in February 1997, and the first cases appeared over a 2-month period in the autumn of 1998, with 30 animals out of a total of 120 exhibiting neurologic defects. A second ‘‘outbreak’’ of cases occurred over a similar time span in the autumn of 1999, with 70 cats affected out of a colony total of 200 SPF animals. A third cluster of cases occurred in the autumn of 2001, when 90 animals out of a colony total of 220 cats presented with ataxia.

Throughout this time, kittens of between 8 and 12 weeks of age that were transferred from the SPF to a conventional disease status colony on the same site did not develop clinical signs. Both SPF and conventional status cats had been fed to appetite on the same commercial formula ration (Gilbertson and Page Ltd., Welwyn Garden City, UK), except that the ration fed to the SPF cats had been irradiated by a single-exposure gamma-radiation treatment between 36.3 and 47.3 kGy (Cobalt 60 irradiator; Isotron Ireland, Tullamore, Ireland). The irradiated diet was consumed to the same extent as the non-irradiated diet, and affected animals did not lose weight until the developing ataxia hindered their access to food. Dietary constituents were determined prior to and after gamma-irradiation treatment.

During the second cluster of cases, 8 out of 70 affected SPF cats (5 male and 3 female) between 12 and 18 months of age, which had been fed gamma-irradiated diet (36.3 and 47.3 kGy), were subjected to full neurologic assessment. The cats exhibited progressive hind ataxia for between 4 and 8 weeks and revealed hind limb ataxia with impaired postural reactions of both hind limbs. There were no changes in haematological parameters and clinical chemistry values, urinalyses or cytological examination of cerebrospinal fluid. Histopathologic examination revealed moderate to severe bilateral axonal degeneration within white matter regions of the cervical, thoracic, and lumbar spinal cord, and in the white matter of the cerebral internal capsule and peduncle, in the roof of the fourth ventricle and inferior cerebellar peduncle, and in the external arcuate and pyramidal fibres of the medulla. There were varying degree of accompanying microgliosis, astrocytosis, and capillary hyperplasia. Following supplementation of the irradiated diet with pasteurized proprietary tinned cat feed in the winter of 2001, and ultimately, the replacement of the irradiated diet with an equivalent pasteurised diet, no further cases occurred.

Another outbreak occurred in Australia between June 2008 and March 2009 (Child *et al.*, 2009). 87 domestic cats developed symmetrical hindlimb ataxia, paraparesis, tetraparesis, paraplegia or tetraplegia in association with exposure to a gamma-irradiated dry cat feed (≥ 50 kGy) imported into Australia in the months preceding the onset of neurological signs in both male and female animals. The diet was subjected to gamma irradiation upon entry to Australia at levels greater than or equal to 50 kGy. The cats had eaten the imported dry food, sold under the brand name Orijen and made in Canada by Champion Petfoods. The feeding period ranged from less than 1 month to 10 months. The onset of clinical signs in all cats appeared at least 2.5 to more than 6 months after they were exposed

to this diet. It is reported that some had eaten the feed for as few as three weeks and others for more than six months. Most of the cats were fed other feeds, as well. In households where cats were affected after eating Orijen dog food, dogs in the household did not show any neurological abnormalities.
Sixty nine affected cats ranging from 10 months to 15 years old were analysed for their neurobehaviour. The initial abnormalities were characterized by difficulty in jumping, landing heavily and a swaying, often wide-based, crouching hind limb gait. Symmetrical hind limb paresis and ataxia progressed in more than 50 of the affected cats, to non-ambulatory severe paraplegia, and in 13 cases to tetraplegia over a period of 4 to 8 weeks. The most affected cats showed tetraparesis and either spastic paraplegia or tetraplegia. More than half remained paraplegic or tetraplegic for months, and more than 25 were euthanised because of the severity of neurological signs. The neurological abnormalities are indicative of an upper motor neuron abnormality with decreased or absent postural reactions in affected limbs, normal to increased spinal reflexes and normal to markedly increased muscle tone. The most severely affected cats showed a subtle head tremor and/or difficulty eating (coordination the prehension of feed), constant liking of the nose, but all have remained seemingly alert and responsive to their owners. No gross abnormalities were found and the histopathological abnormalities were confined to the nervous system in six cats. The study authors remarked that the total amount of irradiated fed diet has not been correlated with the severity of clinical signs. The neuropathologic findings consisted of severe diffuse leukoencephalopathy. Degenerative changes were detected in the spinal cord, with widespread white matter vacuolization. The brain stem and cerebrum were also affected. The demyelination was the predominant feature. No specific treatment has resulted in an improvement in the outcome of affected cats. A change in diet, nursing care, physiotherapy and time seem to be the only factors common in recovered cats.

5.5.2 Experimental studies in laboratory cats

Caulfield *et al.* (2009) tried to induce experimentally the disease by long-term feeding SPF cats with a gamma-irradiated diet. Thirty-one clinically and neurologically normal, male SPF cats of between 6 and 14 months of age and between 3.1 to 6.6 kg bw were randomized into 4 treatment groups. The cats were fed a normal diet (control = 7 animals), group 2 (n=8 animals) was fed a diet gamma-irradiated with typical doses (25.7-38.1 kGy) and groups 3 and 4 (n=8 animals each) were fed a diet gamma-irradiated with high-end doses (38.1-53.6 kGy) for up to 224 days. Cats fed exclusively on irradiated diet developed typical lesions associated with LEM, progressively severe ataxia between study days 140 and 174. Ataxic cats exhibited varying degrees of Wallerian degeneration in the spinal cord and brain, similar to the spontaneous disease. No other abnormality regarding histopathology than in the brain and the spinal cord was observed. Clinical chemistry, haematology or urinalysis did not show any difference between control and treated cats.

Euthanised cats were analysed for tissue total antioxidant and superoxide dismutase of brain and spinal cord. There was a significant difference in the spinal cord segment of tissue total antioxidant between cats from the control group and group 2 at 146-174 and 188-224 days of the study for lumbar and for cervical and lumbar segments, respectively. There was no significant difference in the tissue total antioxidant of spinal cord segments between the control group and groups 3 and 4 cats at the early euthanasia (93-100 days).
In the same study the authors analysed the dietary constituents and the nutritional composition of the diets. They found that the peroxide concentrations of the irradiated diets were 10 fold- (25.7-38.1 kGy) and 64 fold-higher (38.1-53.6 kGy) than those in the non-irradiated diet. Simultaneously, the vitamin A concentrations were reduced to 6230 IU/kg (43%) and 6900 IU/kg (48%), respectively, compared to the initial content of 14,500 IU/kg. These changes in peroxide concentrations may have resulted in increased oxidative insult, a factor probably exacerbated by the observed reduced vitamin A content in the irradiated diets. From this study it appears that cats exhibit these neurological effects when these changes in peroxide and vitamin A concentrations in the diet occur. The elevated total antioxidant status of the spinal cord segment and hepatic superoxide dismutase concentration as well as the increased elevated peroxide concentrations of the irradiated feed suggest free radical involvement in the pathogenesis. However, it is not clearly demonstrated that the vitamin A deficiency

or the increase of peroxides or a combination of these is the cause for these neurological effects associated with LEM.

The same authors investigated the effects of gamma irradiation (doses of 28.9-34.3 kGy and 38.4-48.7 kGy) and of pasteurisation (temperature of 107 °C for 15 min) on the amounts of fat; protein; carbohydrate (and taurine); vitamins A, E, B1, B2, B6, and B12; and peroxides in dry cat, dog, and rodent diets (Caulfield *et al.* 2008). In the dry cat diet the only treatment-related changes occurred with vitamin A and peroxides. Concentration of peroxides in the dry cat diet was increased to 11- and 21-fold after irradiation treatments (28.9–34.3 kGy and 38.4–48.7 kGy), respectively and pasteurisation increased this parameter to 117% of its untreated value. The other dietetic factor such as fat, protein, and carbohydrate levels were not affected by either gamma irradiation dose or by pasteurisation. Taurine levels were only minimally affected by the higher range of irradiation and by pasteurisation, and the values remained well above the minimal levels for cats recommended by the Association of American Feed Control Officials (AAFCO) and the US National Research Council (NRC).

Duncan *et al.* (2009) studied the remyelination of the central nervous system (CNS) in cats. He reported severe neurologic dysfunction, including ataxia, paresis, paralysis, and vision loss, in pregnant cats fed an irradiated diet. Removal of diet results in delayed but complete neurologic recovery associated with extensive remyelination along the entire spinal cord and throughout the optical nerve. Axons remain largely intact; proving that remyelination alone of large areas of the CNS can restore function. Cats were fed an irradiated diet in a feeding trial first in 1995–1996, and then in a more recent trial. Irradiation of different commercially available diets was carried out by the SteriGenics Radiation Facility (Schaumburg, IL) with a minimum or maximum dose of 25.0 - 50.0 kGy. This diet was being tested for nutritional content in pregnant and lactating cats, but the breeding males and offspring were also fed the same diet. Pregnant cats were maintained throughout gestation and lactation. Analysis of the irradiated diet for macronutrient, minerals, vitamins, and fatty acids compared with non-irradiated diets showed no significant differences. Likewise, sampling of tissue from affected cats, which were analysed for various vitamins, heavy metals, parathyroid hormone, insulin, ionized calcium, bacterial endotoxins, aflatoxins, and taurine, was all within normal limits. Clinical chemistry examination from affected cats (hematology, blood biochemistry, and urinalysis) was all within normal limits. A final feeding trial of 2 irradiated commercial diets compared with the same diets that were autoclaved (15 cats per group) showed that approx. 90–95% of pregnant cats on the irradiated diets developed neurologic disease; those that ate more diet developed disease earlier and more severely. The symptoms took about 4 months to appear, and they abated after another 2 to 4 month once the cats were given non-irradiated diet. Non-pregnant female cats, males and the offspring of females exposed to the same diet that developed disease post-parturition never developed neurologic disease. Only cats that became pregnant while on the irradiated diet developed neurological symptoms. Even affected cats who were paralysed and lose vision as a result of eating irradiated diet could fully recover, given sufficient time, nursing care and feed that had not been irradiated.
Attempts to reproduce a similar disorder in pregnant rats fed with irradiated diet were unsuccessful.

5.6 Human clinical studies

No new studies were reported after the last SCF opinion.

6 Allergenicity

According to recent publications, the food irradiation technology has been shown to reduce allergenic properties of some foods tested *in vitro* with human sera. A study carried out to evaluate the reduction of milk allergies through the use of gamma irradiation at doses up to 10 kGy was performed (Lee *et al.*, 2001b). The milk proteins used for the assay were bovine α-casein (ACA) and β-lactoglobulin (BLG) and the binding abilities of milk-hypersensitivepatients' immunoglobulin E (IgE) and rabbit IgGs individually produced to ACA and BLG were determined by competitive indirect ELISA. The

results showed that both proteins were structurally changed with different allergeniciy and antigenicity and that aggregation of the molecules might mask the epitopes on the proteins (Lee *et al.*, 2001b).

A study by Byun (Byun *et al.*, 2002), evaluated the application of gamma-irradiation for inhibition of food allergies with β-lactoglobulin, ovalbumin and shrimp tropomyosin being used as allergens. Sera were individually obtained from each 20-patients group diagnosed with Ig-E mediated milk, egg or shrimp allergy by exposing each food challenges and/or by a convincing history of acute urticaria or diarrhea after ingestion. The patients' IgE did not respond to the irradiated allergens, depending on the dose, the amount of intact allergens in an irradiated solution was reduced by gamma irradiation. The results of this study showed that epitopes on the allergens were structurally altered by irradiation treatment and IgE did not recognize antigen-determinant sites on allergens. The conformational modification of allergens was caused by gamma irradiation at the shrimp muscle. At above 7 kGy, the binding ability of patients' IgE was below 50% at both fractions.

Lee *et al.* (2005) evaluated the changes of the antigenic and allergenic properties of a hen's egg albumin (ovoalbumin) in white layer cakes containing egg white gamma-irradiated. Ovoalbumin as a standard antigen and allergen and mouse monoclonal anti-chicken egg albumin clone OVA-14 IgG (mouse IgG) for OVA were used. In the study, human sera was obtained from 22 patients (less than 3 years of age; 15 boys and 7 girls) diagnosed with the IgE-mediated egg allergy. Mouse IgG recognized OVA in the cakes with irradiated egg white (10 or 20 kGy) better than that in the control. However, the patients' IgE did not recognize OVA in irradiated samples very well. The detected concentrations of intact OVA in the control significantly decreased in the treatments, when determined by IgE-based ELISA. The results indicate that the antigenicity of the OVA increased, but that the allergenicity was decreased by irradiation and processing.

The influence of gamma-irradiation on the allergenicity of shrimp was assessed by Zhenxing *et al.* (2007). Fifteen patients (18 years old) were recruited, confirmed with having a shrimp allergy; they showed a history of atopic disease, with 67% having a history of asthma, 60% of allergic rhinitis, and 20% of atopic dermatitis. Shrimp protein extracts and shrimp muscles were subjected to irradiation at different doses. The allergenicity of irradiated shrimp protein extracts and shrimp muscle was analysed by immunoblotting and Ci-ELISA using pool sera from 15 patients with Shrimp specific IgE. The irradiated shrimp allergen extracts were compared with irradiated shrimp muscle by SDS-PAGE analysis. Results showed that irradiated shrimp allergen extracts exhibited a significant decrease in allergenicity (measured by immunoblot and Ci-ELISA) with the increase of irradiation dose. However irradiated shrimp muscle exhibited an increase of allergenicity before the irradiation dose reached to 5 kGy (10 kGy measured by ELISA), whereas it tended to decrease significantly when the dose exceeded 10 kGy.

Other studies are contradictory to such reduction in allergenicity. A study by Su *et al.* (2004) evaluated the antigenicity of whole unprocessed almonds, cashew nuts and walnuts after gamma-irradiation (using doses between 1 and 25 kGy) followed by heat treatments such as autoclaving (121°C, 15 psi for 15 and 30min), dry-roasting (138 and 160°C for 30 min each, 168 and 177°C for 12 min each), blanching (100°C for 5 and 10 min), oil roasting (191°C, 1min) and microwave heating (500W for 1 and 3min). Immunoreactivity was determined through ELISA and Western blotting experiments using rabbit polyclonal antibodies raised against each major protein. The results revealed that almond, cashew nut and walnut proteins remained antigenically stable after being exposed to gamma-irradiation alone or followed by various thermal treatments (Su *et al.*, 2004). Furthermore, a more recent study carried out with milk and whey exposed to gamma irradiation at doses up to 10 kGy showed that antigenicity of cow´s milk β-lactoglobulin was not reduced by ionizing radiation (Kaddouri *et al.*, 2008).

7 Toxicological aspects of defined radiolytic products

7.1 2-alkylcyclobutanones (2-ACBs), recent *in vitro* genotoxicity data

The toxic potential of 2-ACBs, formed in fat containing foods during irradiation was evaluated by the SCF in 2002 and 2003 (SCF, 2002 and 2003). The studies of cytotoxicity (assay in colon cancer cells), *in vitro* genetic toxicology (reverse mutation test), and induction of DNA breaks *in vitro* (Comet assay) and *in vivo* (assay for promoter activity in rat colon mucosa cells) available in 2003 were not conclusive and the genotoxic potential of 2-ACBs could not be ruled out by this Committee (SCF, 2003). However, these effects only occurred at cytotoxic concentrations of 2-ACBs, a parameter which often calls into question this type of result. On the other hand, other *in vitro* genetic toxicology studies (Ames test) produced negative results (SCF, 2003). The SCF report concluded that additional *in vitro* and *in vivo* studies (standard gene mutation and chromosomal aberration tests and tests in animals) were necessary in order to come to a conclusion on the genotoxic potential of 2-ACBs.

In 1998 Delincée and Pool-Zobel (Delincée and Pool-Zobel, 1998) studied the genotoxic effects of 2-dDCB *in vitro* using rat and human colon cells. The study determined that 2-dDCB at levels of 0.3 – 1.25 mg/ml induced DNA strand breaks as well as a cytotoxic effect that was concentration related (this study was taken into account in the SCF Opinion 2003). The later study by Delincée *et al.et al.* (2002), however, found no indication of any cytotoxic or genotoxic (using the Comet assay) effects on human colon tumour cell lines, HT 29 and HT29 clone 19A, caused by 2-tDCB at the highest concentration tested of 400 µM (approximately 100 µg/ml; 25, 50, 100, 200 and 400 µM tested, equals approximately 6, 12, 25, 50, 100 µg/ml) after 30 minutes. Only after 1-2 days was any cytotoxic effect observed (Delincée et al, 2002).

2-dDCB was also tested negative in Ames test by Sommers in 2003 (Sommers, 2003) and by Gadgil and Smith in 2004 (Gadgil and Smith, 2004). In addition 2-dDCB tested negative in the yeast DEL (deletion) assay with *Saccharomyces cerevisiae* strain *RS112* for recombination (Sommers and Schiestl, 2004) and *in vitro* in *E. coli* lacZ SF1 with regard to its capacity to increase DNA damage-inducible gene expression and to increase the number of 5-fluorouracil-resistant mutant bacteria (Sommers and Mackay, 2005).

In a study by Sommers (Sommers, 2006) 2-dDCB showed a slight genotoxic potential at the highest concentration (12 µg/ml) tested *in vitro*, with regard to its capacity to induce clastogenic effects (formation of micronuclei) in human TK6 lymphoblasts. However, this effect coincided with relatively high cytotoxicity in the absence and presence of metabolic activation. Nevertheless, since micronuclei are expressed only in living cells differently from chromosomal aberrations the results might be interpreted as demonstrating a genotoxic effect.

In another study primary cells, preneoplastic cells (LT97 adenoma cells) and tumour cell lines (HT29clone2A) of the human colon were exposed *in vitro* to increasing concentrations of 2-dDCB (Knoll *et al.*, 2006). The genotoxicity of 2-dDCB in these cells was evaluated by measuring the DNA breaks (Comet assay) at concentrations of 150, 300, 600, 1 049 and 2 097 µM (approximately 40, 75, 150, 250, 500 µg/ml) and by measuring the induction of chromosomal aberrations (fluorescent *in situ* hybridisation technique), the latter at concentrations of 30, 75 and 150 µM (approximately 8, 20 and 35 µg/ml), in the presence and absence of metabolic activation. In this study, 2-dDCB induced DNA breaks in normal and preneoplastic cell line but the tumour cell line was resistant. Moreover, 2-dDCB induced chromosomal aberrations in the preneoplastic cell line.

In 2007 a study was published by Hartwig *et al.* (2007) on the the cyto- and genotoxic potentials of various 2-ACBs in human cell lines. Genotoxicity was demonstrated mainly by the induction of DNA base lesions in HT 29 stem cells recognized by the Fpg protein as determined by both the Comet assay and the alkaline unwinding procedure. Formation of DNA strand breaks was observed by the alkaline unwinding procedure using HeLa and HT 29 cells but not by the Comet assay. In the case of 2-tDCB and 2-tDeCB, DNA damage only occurred at concentrations (70 µg/ml and 50 µg/ml, respectively)

that were already highly cytotoxic, such that considerable fractions of the cells were no longer viable. The situation was different with 2-dDCB and 2-DCB where DNA damage occurred at non-cytotoxic concentrations. The extent of cytotoxicity and genotoxicity was dependent on chain length (shorter - higher degree of toxicity) and degree of unsaturation of the fatty acid chain (higher toxicity for unsaturated fatty acids).

7.2 2-alkylcyclobutanones (2-ACBs), recent *in vivo* data (metabolism)

In 2002, Horvatovich *et al.* (Horvatovich *et al.*, 2002) studied the metabolism of 2-ACBs. Six male Wistar rats received a freshly prepared drinking fluid containing 0.005% unlabelled 2-tDCB- or 2-tDeCB in 1% ethanol daily for 4 months (approximately 1 mg /rat/day were ingested). These two compounds were recovered to a small extent in the adipose tissue determined by gas chromatography-mass spectrometry. Less than 1% of the 2-ACBs ingested daily were excreted in the faeces.

In 2006 Gadgil and Smith (Gadgil and Smith, 2006) carried out a much shorter study on the metabolism of 2-dDCB. Six female Sprague-Dawley rats were administered unlabelled 2-dDCB (5 mg/day) in corn oil for 5 days via gavage. The total amount of 2-dDCB recovered in faeces at the end of 5 days represents between 3 and 11% of the total 2-dDCB administered determined by GC-MS. The total amount recovered in the adipose tissue were ~ 0.33% of the total 2-dDCB administered. No metabolites were recovered in any of the urine extracts.

The results show that at most 11% of the 2-dDCB was recovered unchanged from the faeces and adipose tissue. However, severe shortcomings in these studies, such as use of unlabelled substances, do not allow the investigation of the fate of the substance. Therefore, they cannot contribute to the safety assessment.

7.3 Chronic studies on 2-alkylcyclobutanones (2-ACBs)

Raul *et al.* (2002) reported that 2-ACBs (0.005% wt/vol in drinking water equivalent to a daily intake of 1.6 mg per rat for up to 3 and 6 months) promoted azoxymethane-induced colon carcinogenesis in Wistar rats. In this study, the rats given radiolytic compounds developed significantly more colon tumours than the control group. The reported colon tumour multiplicity (tumour per animal) in control, 2-tDCB, and 2-tDeCB were 0.66, 2.33, and 2.17, respectively. The increase in colon tumours was about 3.5 in animals given 2-tDCB and 2- tDeCB compared with the control group. However, the use of six rats per group and the lack of a control group not given azoxymethane but exposed to the radiolytic agents raise some concerns about the experimental design. It is known that Wistar rats are generally more sensitive to azoxymethane intraperitoneal injection than F344 or Sprague-Dawley rats because the azoxymethane-induced F344 rat model is widely accepted as a more appropriate model to mimic human colon cancer.

Comparing the data by Horvatowich *et al.* (2002) and Raul *et al.* (2002) it can be concluded that there is a clear difference between 2-tDCB and 2-tDeCB in their toxic and tumor promoting potentials. In the study of Horvatovich *et al.* (2002) the retention of 2-tDCB in adipose tissue was more pronounced than that of 2-tDeCB. In the Raul *et al.* study (2002) 2-tDeCB was significantly more effective than 2-tDCB in promoting the appearance of colonic aberrant crypt foci. According to Raul and co-workers, the prolonged accumulation of these compounds in various tissues may give rise to genotoxic effects and/or may possibly induce later on chronic effects, but this has yet to be investigated.

7.4 Other defined radiolytic products

Some products like furans, hydrocarbons and cholesterol oxides have been reported to be generated during irradiation treatment but their formation is not unique to irradiation and they are formed also by conventional heat treatment. Furan is considered as a possible human carcinogen (EFSA, 2004). Fan

(2005b) investigated the formation of furan from carbohydrates and ascorbic acid and in a study with fruit juice following irradiation (Fan, 2005a). Both studies concluded that irradiation does induce the formation of furan although heat treatment has the same effect, producing similar amounts.

None of the long-chain hydrocarbons, pentadecane, tetradecene, heptadecane, hexadecene, heptadecene, hexadecadiene, heptadecadiene, hexadecatriene formed after irradiation of the respective triglycerides palmitic acid, stearic acid, oleic acid and linoleic acid are considered to be of genotoxicological concern from modelling for structural activity relationship. However, hexadecene has been shown to form an epoxide *in vitro* (Watabe and Yamada, 1975).

DISCUSSION

The European Commission asked EFSA to issue an opinion on the safety of the irradiation of certain food products. Taking into consideration the previous opinions of the SCF on irradiation of food, the EFSA should establish whether the food classes and doses specified in these opinions are still up-to-date to ensure consumer safety.

An extensive literature search was performed covering scientific publications relevant to the task until December 2009 for evaluation. The database was maintained and updated until May 2010.

When considering the literature since 2003, the Panel found that even though there is a large number of publications on food irradiation, the number relevant for evaluating chemical safety is limited.

Since the SCF opinion (SCF, 2003), no new data have emerged on the changes in levels of macro- or micronutrient levels following irradiation of foods. These changes may be minimised if food irradiation takes place at low temperatures and in the absence of oxygen. Whether the changes as reported up to now are relevant with respect to the nutritional value of irradiated foods is beyond the scope of this opinion and outside the remit of the CEF Panel.

The allergenic potential of some proteins appears to be reduced after irradiation treatment by changes in the conformational structure even though some studies show no reduction in allergenicity.

The Panel considered the various terms used for defining radiation dose. When food is irradiated there are three main definitions of dose that can be stated; the maximum dose, the minimum dose and the overall average dose. The maximum dose and minimum dose can be measured directly. The minimum dose received by the food must be greater than the dose required to ensure efficacy of the radiation treatment. The maximum dose must be less than any dose that would render the food unacceptable. Food irradiation facilities are capable of operating with a dose uniformity ratio ($\overline{D}$ max/ $\overline{D}$ min) greater than 1.0 but less than 3.0. In contrast, the overall average dose is not a processing parameter but is a concept once used in Codex standards, and still found in national standards and legislation (*e.g.*, Directive 1999/2/EC). The overall average dose cannot be measured directly but can only be estimated. This is difficult for non-uniform foods that have variable densities and spatial geometries (e.g. whole chicken carcasses). It should be noted that the concept of overall average dose is peculiar to food irradiation and is not used in any other area of irradiation technology (e.g sterilization of medical devices and pharmaceuticals). Since 2003, the Codex Standard no longer uses the concept of overall average dose but instead the minimum and maximum dose. The Panel considers this approach sensible, as the overall average dose cannot be measured directly but only estimated.

EU legislation requires that any irradiated food or food ingredient, even if the latter is present in trace amounts in a non-irradiated compound food, has to be labelled as “irradiated” or “treated with ionizing radiation” in order to allow the consumers an informed choice. Analytical methods to detect radiation treatment have been validated and standardised by European Committee for Standardisation and later accepted as Codex General Methods. They are currently used worldwide by official and private laboratories to check radiation treatment of foods. Since publication of the standard methods further

developments have been made regarding the sensitivity of some of the methods and the range of foods which can be analysed. However, these would have to be validated before they can be standardised.

Currently radiation doses are assigned to general food classes and specific food commodities. The Panel recognises the shortcomings of the current classification as it does not consider the physical state of the food (e.g. fresh or frozen food of the same class, water activity), the different composition of products within a food class (e.g. fat content) and the diversity of food products nowadays available to the consumer (*e.g.*, ready-to-eat foods, sliced meat or cheese).

The formation of several chemical substances as a result of radiation treatment of food has been reported in the scientific literature. They belong e.g. to the classes of hydrocarbons, furans, 2-Alkylcyclobutanones (2-ACBs), cholesterol oxides and aldehydes. Most of these substances are also formed in food that has been subjected to other processing treatments and are thus not exclusively formed by irradiation. Furthermore, the quantities in which they occur in irradiated food are not significantly higher than those being formed in heat treatments. Until recently 2-alkylcyclobutanones were only reported in irradiated food, thereby indicating that they may be unique radiolytic products. In 2008, Variyar *et al.* reported that they had detected 2-dDCB and 2-tDCB in commercial non-irradiated and fresh cashew nut samples, as well as 2-DCB and 2-dDCB in non-irradiated nutmeg samples. The presence of 2-tDCB was also observed in both commercial and irradiated cashew nuts (Variyar *et al.*, 2008). As no further evidence of the natural existence of 2-alkylcyclobutanones has been reported, it would be pertinent to treat these findings with some caution until the results are validated by further experimental work.

The toxic potential of 2-ACBs, formed in fat-containing foods during irradiation was evaluated by the SCF in 2002 and 2003 (SCF, 2002 and 2003). Since the last opinion, additional *in vitro* genotoxic studies have been performed. To date both genotoxic and cytotoxic effects have been shown *in vitro* for 2-ACBs although the majority of genotoxicity studies were negative, such as in the Ames test. Most positive findings in fact concern indicative *in vitro* assays (*i.e.* Comet assay or flow cytometric determination of DNA fragmentation) which are potentially biased by toxicity and which detect effects (mainly single strand breaks) with uncertain toxicological significance, considered not appropriate for hazard identification. Moreover, the mechanism by which ACBs induce such effects is not known, even though the direct interaction with DNA can be ruled out (or at least considered unlikely) in view of the negative results in bacterial assays (Sommers and Mackay, 2005) including the Ames tests (Sommers and Schiestl, 2004; Gadgil and Smith, 2004; Hartwig *et al.* 2007), the most robust assay for the detection of electrophilic reactants. Thus, considering that DNA damage induced *in vitro* by 2-ACBs is usually observed in experimental conditions associated with significant toxicity (Delincée and Pool-Zobel, 1998; Delincée *et al.*, 2002; Knoll *et al.*, 2006) and/or after prolonged exposure (Hartwig *et al.* 2007), it is conceivable that DNA fragmentation may result from indirect mechanisms such as cell membrane damage or oxidative DNA damage. The involvement of indirect mechanism(s) in 2-ACB genotoxicity is also suggested by the sub-linear dose-effect relationship observed in most positive studies, with clearly identifiable negative responses in the low concentration range. The same considerations apply to chromosomal damage, *i.e.* micronuclei (Sommers, 2006) and structural chromosomal aberrations (Knoll *et al.*, 2006) induced by 2-dDCB in some systems *in vitro.*

Interestingly, palmitic acid, one of the most abundant fatty acids in the human diet and precursor of the widely studied 2-dDCB, itself causes oxidative DNA damage, DNA strand breaks, cell membrane damage, necrosis and apoptosis *in vitro*, and is positive in Comet and flow cytometric assays in the same dose range as 2-dDCB (Beeharry *et al.*, 2003; de Sousa *et al.*, 2005; Esteves *et al.*, 2002).

In conclusion the available data indicate that at least some 2-ACBs may be genotoxic *in vitro*. The toxicological relevance of these findings, *i.e.* whether 2-ACBs may represent a genotoxic hazard for humans, is not elucidated due to the absence of proper *in vivo* studies. However, several arguments (see paragraphs above) support the hypothesis that the genotoxic hazard associated with 2-ACBs intake is minimal, if any.

Concerning other radiolytic products no new relevant toxicological studies (genotoxic, subchronic, carcinogenic/chronic, reproduction) are reported.

Since the last SCF Opinion in 2003 several *in vitro* genotoxicity studies (Ames test, chromosome aberrations in mammalian cells, micronucleus test and SOS chromo test) on aqueous and/or alcoholic extracts of irradiated foods and only one *in vivo* study have been published. The Panel considers the results of these studies negative.

Four publications (Cassidy *et al.*, 2007; Caulfield *et al.*, 2009; Duncan *et al.*, 2009; Child *et al.* 2009) relate leukoencephalomyelopathy (LEM) in cats with the feeding of highly irradiated diet. There are controversies regarding the effects of irradiation and why irradiated feed causes LEM in cats. Researchers tried to reproduce the observed effects experimentally in two studies (Caulfield *et al.*, 2009; Duncan *et al.*, 2009). In the Caulfield *et al.* (2009) study the disease was reproduced in male cats. Female cats were not used. Duncan *et al.* (2009) found the symptoms only in pregnant cats, but not in their off-spring, males or non-pregnant cats. Another discrepancy regards the relation between feed intake and the severity of the symptoms. Child *et al.* (2009) attributed the clinical symptoms to the feeding of a certain brand of cat feed (irradiated with doses > 50 kGy). The authors observed that the total amount of this diet fed to domestic cats was not correlated with the severity of clinical signs. In contrast to that, Duncan reported that pregnant cats that ate more of the irradiated diet (25-50 kGy) developed disease earlier and more severely.

One third of the cats (190 out of 540) in a colony were affected (Cassidy *et al.*, 2007), whereas in another experiment all cats fed with irradiated diet became ataxic with the severity of the symptoms in correlation with the period of feeding irradiated diet (Caulfield et al, 2009). Many of the cats that became paraplegic did recover after > 2 months but cats that became tetraplegic did not recover completely. However, all authors reported that the neurologic injury only seen in the cat becomes clinically apparent at ca. 4 months after first exposure to irradiated feed.

No neurological dysfunctions caused by irradiated diet have been reported in species other than cats (*e.g.*, in dogs, mice, rats and humans). The disease has not been observed in dogs fed with the same irradiated diet that cause symptoms in cats (Child et al, 2009), nor was it reproduced in pregnant rats that were fed a high irradiated diet (Duncan et al, 2009). No details in relation to the latter study were reported by the authors.

Changes in the composition of feed after gamma irradiation have been observed (Caulfield *et al.*, 2008). The peroxide content of commercial dry animal diets was increased up to 64-fold after gamma irradiation of 53.6 kGy (Caulfield *et al.*, 2009). The fatty acids of diets are susceptible to oxidation following irradiation. By-products from fatty acid oxidation including peroxides, free radicals and other electrophilic metabolites in the body have the potential to cause tissue damage. Whether these products are responsible for the neurological condition in the cats is unknown.

Based on studies reviewed, no conclusion can be drawn for relevance to humans. Due to the lack of data, it cannot be possible to make a link between the cat LEM disease and possible appearance of this disease in humans where LEM pathophysiology has not been established yet, remaining uncertainties. Further investigation and data in this subject are needed.

It is noted that these neurological effects seen in cats (feed irradiated in the range from 25.7 to 53.6 kGy) were observed at doses that exceeded the normal dose range (1-10 kGy) for food for human consumption. Furthermore, in the experiments cats were entirely fed with an irradiated diet (min dose: 25 KGy) but this is not the case for humans as our diet varies more widely.

CONCLUSION

The formation of several chemical substances as a result of radiation treatment of food has been reported in the scientific literature, mainly belonging to the classes of hydrocarbons, furans, 2-

alkylcyclobutanone, cholesterol oxides, peroxides and aldehydes. Most of these substances are also formed in food that has been subjected to other processing treatments and are thus not exclusively formed by irradiation. Furthermore, the quantities in which they occur in irradiated food were not significantly higher than those being formed in heat treatments. Until recently 2-alkylcyclobutanones were only reported in irradiated food, thereby indicating that they may be unique radiolytic products. However, a recent publication reported the occurrence of some 2-alkylcyclobutanones in commercial non-irradiated fresh cashew nut and nutmeg samples.

Since the SCF (2003) opinion, no new data have emerged on the changes in levels of macro- or micronutrient levels following irradiation of foods. Changes relevant to the nutritional value of irradiated foods are beyond the scope of this opinion and outside the remit of the CEF Panel. The Panel noted the various terms used for defining radiation dose and agrees with the approach of the Codex Standard which no longer uses the concept of overall average dose. Therefore it is considered that the limits should be expressed as maximum dose. In order to convert the overall average dose into a maximum dose the conversion factor should not exceed 1.5 which corresponds to the currently maximum allowed dose uniformity ratio of 3.0.

The present categorisation into food groups might not be appropriate, since it does not consider the physical state of the food (*e.g.*, fresh or frozen food of the same class), the water activity, the different composition of products within a food class (*e.g.*, fat content, unsaturated fatty acids content) and the diversity of food products nowadays available to the consumer (*e.g.*, ready-to-eat foods, sliced meat or cheese).

Concerning the safety assessment of irradiation, the Panel reviewed the recent toxicological studies on alkylcyclobutanones. These studies indicate that at least some alkylcyclobutanones can induce DNA damage *in vitro*. No *in vivo* genotoxicity studies are available; however, a genotoxic hazard in humans is considered unlikely by the Panel in view of the plausible indirect mechanism underlying the genotoxicity of alkylcyclobutanones *in vitro*.

Studies on allergenicity of specific foods after irradiation, covering *in vitro* studies only, show no consistency as regards allergic reactions. Consequently, no conclusion can be drawn on aspects of allergenicity for humans.

The weight of evidence from the existing literature arising from the recent survey of literature regarding biological effects supports the food classes and radiation doses specified in previous SCF opinions. The only new contrary evidence was indicated in publications on leukoencephalomyelopathy in cats which have been fed mainly or exclusively with highly irradiated feed (>25 kGy). This finding has only been reported with cats. In one report, dogs consumed the same pet food and did not show the disease. Several hypotheses have been put forward in the literature (*e.g.*, specific sensitivity of cats to deficiency in vitamins which may be caused by irradiation, peroxides generated by irradiation). However a clear mechanistic explanation in terms of risk assessment has not been established.

Information on the cause and pathogenesis in cats should be collected, including data on the relationship between irradiation dose, composition of feed, the amount of consumed irradiated feed and the elicitation of the leukoencephalomyelopathy. In absence of this understanding, the relevance for humans cannot be ruled out.

Considering that only a very limited quantity of food is irradiated in Europe currently, the Panel is of the view that there is not an immediate cause for concern. However, the relevance of the cats studies for human health should be clarified.

ABBREVIATIONS

AA	Ascorbic acid
AAFCO	Association of American Feed Control Officials
ACA	α-casein
2-ACB	2-Alkylcyclobutanone
AFSSA	French Food Safety Agency
BIOHAZ	Biological Hazards
BLG	β-lactoglobulin
CEF	Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEN	European Committee for Standardisation
CHO	Chinese hamster ovary
CNS	central nervous system
COPs	Cholesterol Oxidation Products
Co-60	Cobalt-60
Cs-137	Caesium-137
Da	Dalton
2-DCB	2-Decylcyclobutanone
2-dDCB	2-Dodecylcyclobutanone
2-tDCB	2-Tetradecylcyclobutanone
2-tDdeCB	2-Tetradeca-5',8'-dienylcyclobutanone
2-tDeCB	2-Tetradecenylcyclobutanone
DEFT/APC	Direct Epifluorescent Filter Technique/Aerobic Plate Count
DHA	Dehydroascorbic acid
Dmax	Maximum dose
Dmin	Minimum dose
EC	European Commission
EFSA	European Food Safety Authority
ELISA	Enzyme-Linked ImmunoSorbent Assay
EN	European Standards
ESR	Electron spin resonance
EU	European Union
FAO	Food and Agriculture Organisation
FDA	Food & Drug Administration
FSTA	Food Science and Technology Abstracts
GC-MS	Gas chromatography mass spectrometry
KGy	Kilogray
HACCP	Hazard Analysis and Critical Control Points
IAEA	International Atomic Energy Association
ICR	Imprinting control region
Ig	Immunoglobulin
LEM	Leukoencephalomyelopathy
LOD	Limit of Detection
OAD	Overall average dose
MeV	Megaelectron Volt
MRI	Max Rubner Institute
MUFA	Monounsaturated fatty acids
NRC	US National Research Council
OECD	Organisation for Economical Cooperation and Development
OVA	Ovoalbumin
PER	Protein efficiency ratio
PSL	Photostimulated fluorescence
PUFA	Polyunsaturated fatty acids

RTE	Ready-to-eat
SCF	Scientific Committee on Food
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SFA	Saturated fatty acids
SFE-TLC	Supercritical Fluid CO2 Extraction and Thin Layer Chromatography
TIA	Trypsin inhibitor activity
TL	Thermoluminiscence
TLC	Thin layer chromatography
UDS	Unscheduled DNA Synthesis
URP	Unique radioactive products
USA	United States of America
WHO	World Health Organisation

GLOSSARY

Absorbed dose (D)	Sometimes referred to simply as 'dose', is the amount of energy absorbed (imparted) per unit mass of irradiated food product (CAC, 2003b)
Dose (D)	See absorbed dose
Dose Limit	The limit set in legislation, which is deemed higher than the lowest dose required to achieve the desired effect but lower or equal to a dose which would severely impair the sensory and functional properties of the food in question. Dose limits can be expressed as ranges or as single lower or upper values (*i.e.*, no part of the food product shall absorb less than or more than a specified amount) (CAC, 2003b). Current European legislation expresses a dose limit in terms of two parameters; a maximum allowed "dose uniformity ratio" and the now redundant concept of "overall average dose". This 10 kGy overall average dose limit for dried aromatic herbs, spices and vegetable seasonings, is therefore equal to a "maximum dose" limit of 15 kGy at the maximum allowed "dose uniformity ratio" of 3.
Dose uniformity ratio (U)	The ratio of maximum to minimum absorbed dose in the production lot (CAC, 2003b), irradiated as a single entity. $U = \overline{D}_{max} \div \overline{D}_{min}$. Small scale irradiation cells used in research may have $U \approx 1.0$, whereas large commercial irradiation facilities typically operate with a $U \leq 3.0$.
Dosimetry	The measurement of the absorbed dose of radiation at a particular point in a given absorbing medium (CAC, 2003b).
G-value	The number of molecules of reactant consumed or product formed per 100 eV of energy absorbed from ionizing radiation.
Gray (Gy)	Unit of absorbed dose where 1 Gy is equivalent to the absorption of 1 joule per kilogram (FAO, 2008).
Irradiation	In this document irradiation refers to treatment with ionizing radiation.
Maximum dose ($\overline{D}_{max}$)	The highest absorbed dose in a production lot irradiated as a single entity
Minimum dose ($\overline{D}_{min}$)	The lowest absorbed dose in a production lot irradiated as a single entity
Overall average dose ($\overline{D}$)	A concept formerly used by the FAO Codex Alimentarius Commission, but no longer used in the revised Codex General Standard for Irradiated Food (CAC, 2003a). Overall average (absorbed) dose is the arithmetic mean of the dose received by every spatial element of the food. It cannot be measured directly but can be estimated, for example with a homogenous food of

uniform density

$\overline{D} \approx$ ½($\overline{D}$ max+ $\overline{D}$ min).

In general:

$$\overline{D} = \frac{1}{M} \int p(x,y,z) d(x,y,z) \, \mathrm{dV}$$

Where:

M = the total mass of the treated food

p = the local density at the point (x,y,z)

d = the local absorbed dose at the point (x,y,z)

dV = dx dy dz, the infinitesimal volume element which in real cases is represented by the volume fractions.

ANNEX

Action of ionisation radiation on food

Gamma ray, X-ray and E-beam radiation give rise to energetic electrons (secondary electrons) in food during irradiation. These secondary electrons then collide with other molecules or electrons in the material and form either energetic molecular ions, and further, electrons that cascade through the food. The effects on the food depends upon a number of factors that include irradiation parameters (type of radiation, its energy, the dose rate and duration of exposure), as well as other factors such as composition and physical state of the food, its temperature and atmospheric environment during irradiation.

Electron beam and gamma rays differ in their ability to penetrate matter and this has important consequences for the dose distribution in the irradiated material. When ionizing radiation (gamma rays, X-rays or E-beams) penetrates into material the dose builds up as secondary electrons are scattered within the target material. Initially there is a slight build-up as the electrons scatter and cascade within the material

Dose distribution with depth

Gamma rays and X-rays

With gamma ray or X-ray photons, the process of energy transfer to the irradiated medium takes place in two distinct stages:
(a) Photons interact with the material and set secondary electrons (high energy electrons) in motion, this transfer takes place on the atomic scale, at the point of interaction.
(b) The secondary electrons transfer energy to the material through excitation and ionization of the atoms of the medium; these transfers take place over a certain distance.

If the incident radiation is essentially monoenergetic and the angle of incidence at the irradiated surface is approximately perpendicular and monodirectional, there is initially a marked increase (build-up) of energy deposition (dose) near the incident surface. This region extends up to the depth corresponding to the average range of the first interaction secondary electrons. This is then followed by an exponential decay of dose to greater depths. The approximate build-up depth, in units of millimetres of water, is given below for different photon energies,

Cs-137 gamma rays ≈ 3 mm of water (*i.e.* the maximum dose is 3 mm below the incident surface)
Co-60 gamma rays ≈ 5 mm of water
4 MeV X-rays ≈ 10 mm of water
6 MeV X-rays ≈ 16 mm of water
10 MeV X-rays ≈ 30 mm of water

However, if the incident photon energy spectrum is fairly broad (*e.g.*, for X rays) or the angles of incidence are widely varying (*e.g.*, gamma ray sources in commercial irradiation facilities where the Co-60 is arranged in a rack (plaque source) of extended size close to the absorbing material), there is no appreciable dose build-up region. With a diffuse broad beam incidence of photons, there is, instead, a pseudo-exponential decrease in dose with depth due to attenuation beginning at the incidence surface. The shape of the depth–dose distribution in the irradiated material depends on a number of factors; including the source geometry, source-to-material distance and the geometry of the irradiated material.

E-Beams

With incident electron beams that are used in food processing there is generally a build-up region in low atomic number materials due to the progressive cascading of secondary electrons and collision energy transfers. This build-up region extends up to a depth of approximately one third to two thirds of the electron range, except when the angle of incidence is greatly increased (away from the normal)

or when a scattering material is placed between the source and the irradiated material. The approximate value of the build-up depth, in units of millimetres of water, is given below for a 10 MeV electron beam.

10 MeV E-beam ≈ 30.1 mm of water (*i.e.* the maximum dose is 30.1 mm below the incident surface)

With a scanned E-beam the angle of incidence can vary by about ±15° and thus the resulting depth–dose distribution will vary as a function of the incident angle. Also, the electron beam may also experience scattering effects which serve to reduce the build-up region and reduce the depth where the dose reaches a maximum value.

The difference between gamma ray (or X-ray) and E-Beam irradiation is that with gamma rays in a commercial irradiation facility the maximum dose is effectively at the surface of the food where the radiation is incident. However, with E-Beam facilities the maximum dose is located beneath the surface where the E-Beam is incident.

Typical dose distributions in irradiated products

In an irradiation facility, the dose uniformity ratio can be optimised, for example by irradiating a product in different orientations so that the ionising radiation is incident on different sides of the process load. A conveyor system that makes multiple passes through a gamma irradiator or through an electron beam is one way of ensuring that ionizing radiation is incident on different sides of the process load.
Figure A is a diagram to show the dose-depth distribution for a two pass gamma irradiation facility, where radiation is incident on first one side of the process load and then on the opposite side of the process load on the second pass. Note that the dose distribution is symmetrical with the minimum dose located at the centre of the process load and the location of the maximum dose is effectively at the surface.
Figure B illustrates E-beam dose-depth distributions for different thicknesses of water irradiated on two sides (the dose is normalised to a surface dose of 100%). Note that the dose distribution is symmetrical but more complicated and the maximum dose is not located at the surface of the process load.

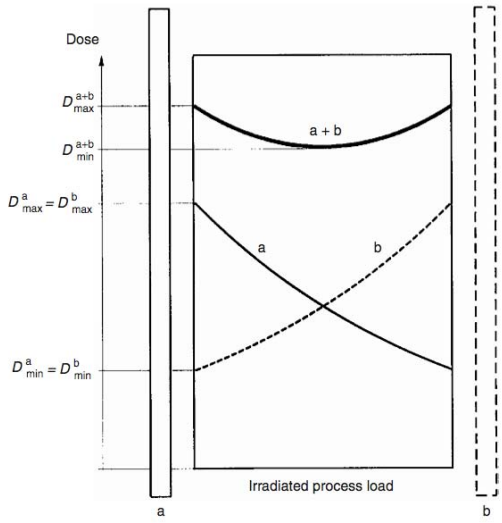

Figure A. Depth–dose curves in a process load irradiated from two sides with a Co-60 (gamma ray) plaque source. During the first pass the source is on the side 'a' relative to the process load, while during the second pass the source is on the side 'b' relative to the process load. The curves a and b represent the dose contributions for the first and second passes separately, and the curve a + b represents the accumulated dose from both the passes. (Dosimetry for food irradiation. Technical reports series No. 409, IAEA Vienna (2002)).

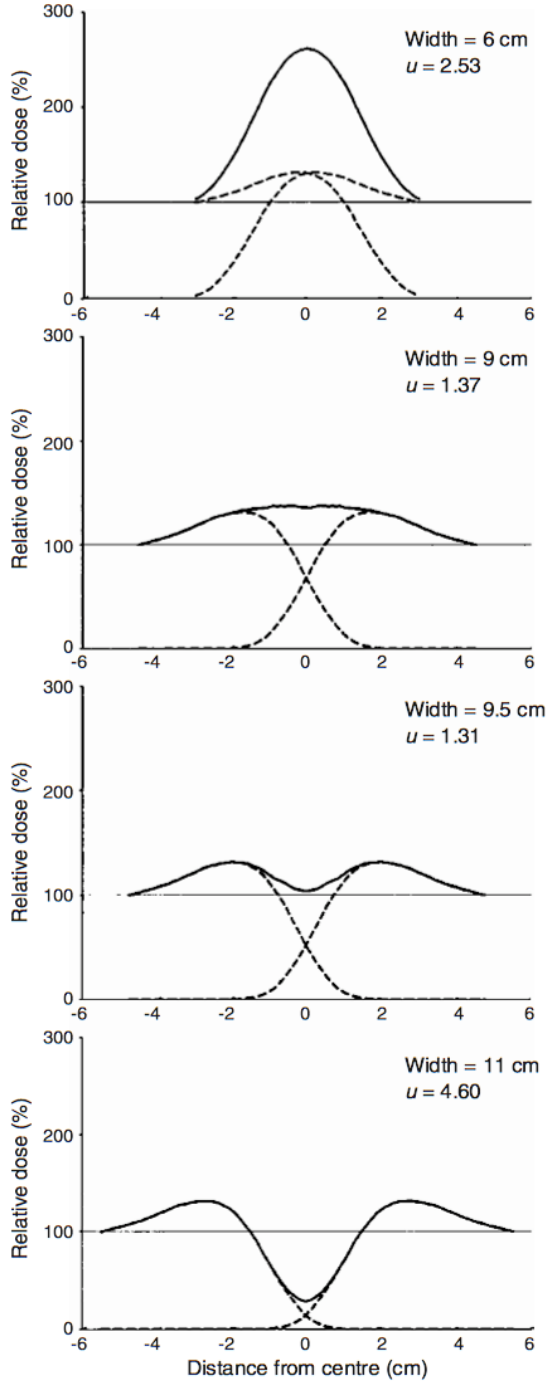

Figure B. Depth–dose distributions for 10 MeV electrons in varying thicknesses (widths) of water absorber; the dashed curves represent the distributions for one sided irradiation from each side, and the solid curves represent the sum of the two distributions. The dose uniformity ratio, u, varies with the absorber thickness, going through a minimum value of 1.31 at about 9.5 cm of water. (Note: The fine horizontal lines represent the surface dose for one sided irradiation normalized to 100%) (Dosimetry for food irradiation. Technical reports series No. 409, IAEA Vienna (2002)).

REFERENCES

Adam S., 1983. Recent developments in radiation chemistry of carbohydrates. In: Recent advances in food irradiation (P.S. Elias and A.J. Cohen, Eds.), Elsevier Biomedical Press, Amsterdam, The Netherlands, pp. 149-170.

AFFSA, 2007. Review of recent data on the ionising radiation of foodstuffs intended for human consumption. AFFSA, French Food Agency, April 2007.

Ahn HJ, Jo C, Kim JH, Chung YJ, Lee CH and Byun MW, 2002. Monitoring of nitrite and N-nitrosamine levels in irradiated pork sausage. Journal of Food Protection, 65, 1493-1497.

Ahn HJ, Kim JH, Jo C, Lee JW, Yook HS and Byun MW, 2004. Effects of gamma irradiation on residual nitrite, residual ascorbate, color, and N-nitrosamines of cooked sausage during storage. Food Control, 15, 197-203.

Araujo MM, Marin-Huachaca NS, Mancini-Filho J, Delincée H and Villavicencio ALCH, 2004. Identification of irradiated refrigerated pork with the DNA Comet assay. Radiation Physics and Chemistry, 71, 183-185.

Ashley BC, Birchfield PT, Chamberlain BV, Kotwal RS, McClellan SF, Moynihan S, Patni SB, Salmon SA and Au WW, 2004. Health concerns regarding consumption of irradiated food. International Journal of Hygiene and Environmental Health, 207, 493-504.

Barba C, Calvo MM, Herraiz M and Santa-Maria G, 2009. Detection of radiolytic hydrocarbons by supercritical fluid extraction and gas chromatographic-mass spectrometric analysis of irradiated cheese. Food Chemistry, 114, 1517-1522.

Bayram G and Delincée H, 2004. Identification of irradiated Turkish foodstuffs combining various physical detection methods. Food Control, 15, 81-91.

Beeharry N, Lowe JE, Hernandez AR, Chambers JA, Fucassi F, Cragg PJ, Green MHL and Green IC, 2003. Linoleic acid and antioxidants protect against DNA damage and apoptosis induced by palmitic acid. Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis, 530, 27-33.

Bhaskar S, Sajilata MG, Suchandra C, Rekha SS, Prasad SV, Kamat MY and Arun S, 2008. Identification of irradiated cashew nut by electron paramagnetic resonance spectroscopy. Journal of Agricultural and Food Chemistry, 56, 8987-8991.

Bhattacharjee P, Singhal RS, Gholap AS, Variyar PS and Bongirwar DR, 2003. Hydrocarbons as marker compounds for irradiated cashew nuts. Food Chemistry 80, 151-157.

Blanch GP, Caja MD, Flores G and del Castillo MLR, 2009. Identification of 2-dodecylcyclobutanone and linear-alkanes as irradiation markers in sliced dry-cured ham. Food Chemistry 113, 616-620.

Boniglia C, Aureli P, Bortolin E and Onori S, 2009. Verification of imported food upon import for radiation processing: Dried herbs, including herbs used in food supplements, and spices by PSL and TL. Radiation Physics and Chemistry, 78, 679-681.

Bortolin E, Boniglia C, Gargiulo R and Onori S, 2009. Herbal materials used in dietary supplements: Comparison of luminescence methods for detection of irradiation. Radiation Physics and Chemistry 78, 683-685.

Bortolin E, Bustos Griffin E, Cruz-Zaragoza E, Coste V and Onori S, 2006. Electron paramagnetic resonance detection of Mexican irradiated spices. International Journal of Food Science and Technology 41, 375-382.

Breitfellner F, Solar S and Sontag G, 2003. Radiation induced chemical changes of phenolic compounds in strawberries. Radiation Physics and Chemistry 67, 497-499.

Brigide P and Canniatti-Brazaca SG, 2006. Antinutrients and '*in vitro*' availability of iron in irradiated common beans (*Phaseolus vulgaris*). Food Chemistry 98, 85-89.

Brito MS, Villavicencio ALCH and Mancini J, 2002. Effects of irradiation on trans fatty acids formation in ground beef. Radiation Physics and Chemistry 63, 337-340.

Byun MW, Lee JW, Yook HS, Jo CR and Kim HY, 2002. Application of gamma irradiation for inhibition of food allergy. Radiation Physics and Chemistry 63, 369-370.

CAC (Codex Alimentarius Commission), 2003a. Revised codex general standard for irradiated foods (CODEX STAN 106-1983, REV.1-2003).

CAC (Codex Alimentarius Commission), 2003b. Recommended international code of practice for radiation processing of food (CAC/RCP 19-1979, Rev. 2-2003).

Codex Alimentarius, 1979. Recommended international code of practice for irradiation processing of food. CAC/RCP 19-1979. Rev. 2-2003.

Codex Alimentarius, 1983. Codex general standard for irradiated food. CX/STAN 106-1983, Rev. 1-2003.

Caja MM, del Castillo MLR and Blanch GP, 2008. Solid phase microextraction as a methodology in the detection of irradiation markers in ground beef. Food Chemistry 110, 531-537.

Calucci L, Pinzino C, Zandomeneghi M, Capocchi A, Ghiringhelli S, Saviozzi F, Tozzi S and Galleschi L, 2003. Effects of gamma-irradiation on the free radical and antioxidant contents in nine aromatic herbs and spices. Journal of Agricultural and Food Chemistry 51, 927-934.

Cassidy JP, Caulfield CD, Jones BR, Worrall, S, Conlon L, Palmer AC and Kelly J, 2007. Leukoencephalomyelopathy in specific pathogen-free cats. Veterinary Pathology 44, 912-916.

Caulfield CD, Cassidy JP, Kelly JP, 2008. Effects of Gamma Irradiation and Pasteurizationon the Nutritive Composition of Commercially Available Animal Diets. Journal of the American Association for Laboratory Animal Science 47, 61-66.

Caulfield CD, Kelly JP, Jones BR, Worral S, Conlon L, Palmer AC, Cassidy JP, 2009. The Experimental Induction of Leukoencephalomyelopathy in Cats. Veterinary Pathology 46, 1258-1269.

Chawla SP and Thomas P, 2004. Identification of irradiated meat using electron spin resonance spectroscopy: results of blind trials. International Journal of Food Science and Technology, 39, 653-660.

Child G, Foster DJ, Fougere BJ, Milan JM, Rozmanec M, 2009. Ataxia and paralysis in cats in Australia associated with exposure to an imported gamma-irradiated commercial dry pet food. Australian Veterinary Journal 87, 349-351.

Choi ID, Byun MW, Kwon JH and Kim DH, 2004a. An application of photo-stimulated luminescence (PSL) and electron spin resonance (ESR) analysis for the irradiated spicy vegetables. Food Science and Biotechnology 13, 646-650.

Choi ID, Kang EK and Yang JS, 2004b. Irradiated foods detection by thermoluminescence (TL) and DEFT/APC analysis. Food Science and Biotechnology 13, 151-155.

Chung HW, Hong JH, Kim MC, Marshall MR, Jeong Y and Han SB, 2004. Detection properties of irradiated ostrich meat by DNA Comet assay and radiation-induced hydrocarbons. Journal of Food Science 69, C399-C403.

da Trindade RA, Mancini J and Villavicencio A, 2009. Effects of natural antioxidants on the lipid profile of electron beam-irradiated beef burgers. European Journal of Lipid Science and Technology 111, 1161-1168.

de Sousa ULJ, Koss MD, Fillies M, Gahl A, Scheeder MRL, Cardoso MC, Leonhardt H, Geary N, Langhans W and Leonhardt M, 2005. CPT1 alpha over-expression increases long-chain fatty acid oxidation and reduces cell viability with incremental palmitic acid concentration in 293T cells. Biochemical and Biophysical Research Communications 338, 757-761.

Delincée H, 1983a. In: *Recent advances in food irradiation*; Elias PS and Cohen AJ; Eds; Elsevier Biomedical Press: Amsterdam; pp: 89-114.

Delincée H, 1983b. In: *Recent advances in food irradiation*; Elias PS and Cohen AJ; Eds; Elsevier Biomedical Press: Amsterdam; pp: 129-147.

Delincée H and Pool-Zobel BL, 1998. Genotoxic properties of 2-dodecylcyclobutanone, a compound formed on irradiation of food containing fat. Radiation Physics and Chemistry, 52, 39-42.

Delincée H, Soika C, Horvatovich P, Rechkemmer G and Marchioni E, 2002. Genotoxicity of 2-alkylcyclobutanones, markers for an irradiation treatment in fat-containing food - Part I: Cyto- and genotoxic potential of 2-tetradecylcyclobutanone. Radiation Physics and Chemistry 63, 431-435.

Diehl, JF 1991 Nutritional effects of combining irradiation with other treatments. Food Control 2, 20-25.

Diehl JF, Hasselmann C and Kilcast D 1991. Regulation of food irradiation in the European Community: is nutrition an issue? Food Control 2, 212-219.

Diehl JF 1992. Food irradiation: is it an alternative to chemical preservatives? Food Additives and Contaminants 9, 409-416.
Diehl JF, 1995. Safety of irradiated foods. Marcel Dekker, N.Y.
Dogan A, Siyakus G and Severcan F, 2007. FTIR spectroscopic characterization of irradiated hazelnut (Corylus avellana L.). Food Chemistry 100, 1106-1114.
Duliu OG, Ferdes M and Ferdes OS, 2004. EPR study of some irradiated food enzymes. Journal of Radioanalytical and Nuclear Chemistry 260, 273-277.
Duncan ID, Brower A, Kondo Y, Curlee JF, and Schultz RD, 2009. Extensive remyelination of the CNS leads to functional recovery. PNAS 106, 6832-6.
EC (European Commission), 2002. Report from the Commission on food irradiation for the period September 2000 to December 2001, OJ C 255/02. 2-12.
EC (European Commission), 2004. Report from the Commission on food irradiation for the year 2002. 1-17.
EC (European Commission), 2006a. Report from the Commission on food irradiation for the year 2003, OJ C 230/07. 12-27.
EC (European Commission), 2006b. Report from the Commission on food irradiation for the year 2004, OJ C 230/08. 28-45.
EC (European Commission), 2007. Report from the Commission on food irradiation for the year 2005, OJ C 122/03. 3-21.
EC (European Commission), 2008. Report from the Commission on food irradiation for the year 2006, OJ C 282/04. 3-19.
EC (European Commission), 2009. Report from the Commission on food irradiation for the year 2007, OJ C 242/02. 2-18.
EFSA Panel on EFSA Panel on Biological Hazards (BIOHAZ) 2011. Scientific Opinion on the efficacy and microbiological safety of irradiation of food The EFSA Journal 2011, 9(4); 2103.
El-Niely HFG, 2007. Effect of radiation processing on antinutrients, in-vitro protein digestibility and protein efficiency ratio bioassay of legume seeds. Radiation Physics and Chemistry 76, p.1050-1057.
Engin B, 2004. Use of thermoluminescence technique for the detection of irradiated spices. Journal of Food Science and Technology-Mysore 41, 368-373.
Engin B, 2007. Thermoluminescence parameters and kinetics of irradiated inorganic dust collected from black peppers. Food Control 18, 243-250.
Erel Y, Yazici N, Ozvatan S, Ercin D and Cetinkaya N, 2009. Detection of irradiated quail meat by using DNA Comet assay and evaluation of comets by image analysis. Radiation Physics and Chemistry 78, 776-781.
Esteves MP, Raymundo A, De Sousa I, Andrade ME and Empis J, 2002. Rheological behaviour of white pepper gels - A new method for studying the effect of irradiation. Radiation Physics and Chemistry 64, 323-329.
Fan X, 2003. Ionizing Radiation Induces Formation of Malondialdehyde, Formaldehyde, and Acetaldehyde from Carbohydrates and Organic Acid, Journal of Agricultural and Food Chemistry 51, 5946–5949.
Fan X, 2005a. Impact of ionizing radiation and thermal treatments on furan levels in fruit juice. Journal of Food Science 70.
Fan XT, 2005b. Formation of furan from carbohydrates and ascorbic acid following exposure to ionizing radiation and thermal processing. Journal of Agricultural and Food Chemistry 53, 7826-7831.
Fan X, 2007. Control of irradiation-induced lipid oxidation and volatile sulfur compounds using antioxidants in raw meat and ready-to-eat meat products. Antioxidant Measurement and Applications, Chapter 26, pp 401- 418.
Fan X and Mastovska K, 2006. Effectiveness of ionizing radiation in reducing furan and acrylamide levels in foods. Journal of Agricultural and Food Chemistry 54, 8266-8270.
Fan XT and Sommers CH, 2006. Effect of gamma radiation on furan formation in ready-to-eat products and their ingredients. Journal of Food Science 71, C407-C412.

Fan X and Thayer DW, 2002. Formation of malonaldehyde, formaldehyde, and acetaldehyde in apple juice induced by ionizing radiation. Journal of Food Science 67, 2523-2528.

Fan X, Thayer DW and Handel AP. 2002 Nutritional quality of irradiated orange juice. Journal of Food Processing Preservation 26, 195-211.

FAO (Food and Agricultural Organization), 2003. International standards for phytosanitary measures. Guidelines for the use of irradiation as a phytosanitary measure. Publication No. 18 (2003).

FAO (Food and Agricultural Organization), 2008. International standards for phytosanitary measures (ISPM). International Standards for Phytosanitary Measures (ISPM) no. 5, Glossary of phytosanitary terms.

FAO (Food and Agricultural Organization), 2009. International standards for phytosanitary measures. Guidelines for the use of irradiation as a phytosanitary measure. Publication No. 28 (2009).

Farkas J, Andrassy E, Mészáros L, Polyák-Fehér Beczner J, Gaál O, Lebovics VK and Lugasi A, 2009. Part II: Effects of gamma irradiation on lipid and cholesterol oxidation in mecahnically deboned turkey meat. In: Irradiation to ensure the safety and quality of prepared meals. Results of the Coordinated Research Project organised by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture (2002-2006). IAEA, Vienna, p 95-102.

FDA (United States Food and Drug Administration), 1986. Irradiation in the production, processing, and handling of food; final rule. Federal register, 51 FR 13375- 13399, 18 April 1986.

FDA (Food and Drug Administration), 1987. Federal Register, 52, 6391.

FDA (Food and Drug Administration), 1991. Irradiation in the production, processing and handling of food. Code Federal Register, Title 21, part 179.

FDA (Food and Drug Administration), 2005. Irradiation in the Production, Processing, and Handling of Food. Department of Health and Human Services. FDA. 21 CFR Part 119. Federal Register Vol. 70, No. 157. August 16 2005.

Fielding L (Food Standards Agency), 2008. The Safety of Irradiated Foods: A literature review. Project A05009

Follett PA, 2004. Irradiation to control insects in fruits and vegetables for export from Hawaii. Radiation Physics and Chemistry 71, 161-164.

Fox JB, Thayer DW, Jenkins RK, Phillips JG, Ackerman SA, Beecher GR, Holden JM, Morrow FD and Quirbach DM, 1989. Effect of gamma-irradiation on the B-vitamins of pork chops and chicken breasts. International Journal of Radiation Biology 55, 689-703.

Gadgil P, Hachmeister KA, Smith JS and Kropf DH, 2002. 2-Alkylcyclobutanones as irradiation dose indicators in irradiated ground beef patties. Journal of Agricultural and Food Chemistry 50, 5746-5750.

Gadgil P and Smith JS, 2004. Mutagenicity and acute toxicity evaluation of 2-dodecylcyclobutanone. Journal of Food Science, 69, 9, C713-C716.

Gadgil P and Smith JS, 2006. Metabolism by rats of 2-dodecylcyclobutanone, a radiolytic compound present in irradiated beef. Journal of Agricultural and Food Chemistry 54, 4896-4900.

Gadgil P, Smith JS, Hachmeister KA and Kropf DH, 2005. Evaluation of 2-dodecylcyclobutanone as an irradiation dose indicator in fresh irradiated ground beef. Journal of Agricultural and Food Chemistry 53, 1890-1893.

Galan I, Garcia ML and Selgas MD, 2010. Effects of irradiation on hamburgers enriched with folic acid. Meat Science 84, 437-443.

Gardner LK and Lawrence GD. 1993. Benzene production from decarboxylation of benzoic acid in the presence of ascorbic acid and a transition-metal catalyst. Journal of Agricultural and Food Chemistry 41, 693-695.

Geissler C, Brede O and Reinhardt J, 2003. cis-trans-isomerization of unsaturated fatty acids during gamma-irradiation of barley grains. Radiation Physics and Chemistry 67, 105-113.

Hagiwara A, Yoshino H, Sano M, Kawabe M, Tamano S, Sakaue K, Nakamura M, Tada M, Imaida K and Shirai T, 2005. Thirteen-week feeding study of thaumatin (a natural proteinaceous sweetener), sterilized by electron beam irradiation, in Sprague-Dawley rats. Food and Chemical Toxicology 43, 1297-1302.

Hanis T, Jelen P, Klir P, Mauková J, Pérez B and Pesek M. 1988. Effecto of gamma irradiation on survival of natural micro flora and some nutrients in cereal meals. Cereal Chemistry 65, 381-383.

Hartwig A, Pelzer A, Burnouf D, Titéca H, Delincée H, Briviba K, Soika C, Hodapp C, Raul F, Miesch M, Werner D, Horvatovich P and Marchioni E, 2007. Toxicological potential of 2-alkylcyclobutanones - specific radiolytic products in irradiated fat-containing food - in bacteria and human cell lines. Food and Chemical Toxicology 45, 2581-2591.

Hein WG, Simat TJ and Steinhart H, 2000. Detection of irradiated food - Determination of non-protein bound o-tyrosine as a marker for the detection of irradiated shrimps. European Food Research and Technology 210, 299-304.

Hendricks WH, Allan FJ, Tarttelin MF, Collett MG, Jones BR, 2001. Suspected zinc-induced copper deficiency in growing kittens exposed to galvanized iron. NZ Vet Journal 49, 68-72.

Hong-Sun Y, Bo-Sook C, Dong-Ho K, Ju-Woon L and Myung-Woo B, 2004. Genotoxicological safety of gamma-irradiated salted and fermented anchovy sauce. Journal of the Korean Society of Food Science and Nutrition, 33, 1192-1200.

Horvatovich P, Miesch M, Hasselmann C, Delincée H and Marchioni E, 2005. Determination of monounsaturated alkyl side chain 2-alkylcyclobutanones in irradiated foods. Journal of Agricultural and Food Chemistry 53, 5836-5841.

Horvatovich P, Raul F, Miesch M, Burnouf D, Delincée H, Hartwig A, Werner D and Marchioni E, 2002. Detection of 2-alkylcyclobutanones, markers for irradiated foods, in adipose tissues of animals fed with these substances. Journal of Food Protection 65, 1610-1613.

Hwang KT, Kim JE, Park JN and Yang JS, 2007. Effects of roasting, powdering and storing irradiated soybeans on hydrocarbon detection for identifying post-irradiation of soybeans. Food Chemistry 102, 263-269.

Hyun-Ja L, Kun-Ok K and Hong-Sun Y, 2001. In vitro genotoxicological safety of fresh vegetable-extract juice by gamma irradiation. Journal of the Korean Society of Food Science and Nutrition 30, 1227-1236.

Il-Jun K, Young-Hee K, Cha-Kwon C, Sung-Hoon O, Ju-Woon L and Myung-Woo B, 2005. Genotoxicological safety of high-dose irradiated porridges. Journal of the Korean Society of Food Science and Nutrition, 34, 261-266.

Jo C, Ahn HJ, Son JH, Lee JW and Byun MW, 2003. Packaging and irradiation effect on lipid oxidation, color, residual nitrite content, and nitrosamine formation in cooked pork sausage. Food Control 14, 7-12.

Jo D and Kwon JH, 2006. Detection of radiation-induced markers from parts of irradiated kiwifruits. Food Control 17, 617-621.

Johnsson L and Dutta PC, 2006. Determination of phytosterol oxides in some food products by using an optimized transesterification method. Food Chemistry 97, 606-613.

Kaddouri H, Mimoun S, El-Mecherfi KE, Chekroun A, Kheroua O and Saidi D, 2008. Impact of alpha-radiation on antigenic properties of cow's milk beta-lactoglobulin. Journal of Food Protection 71, 1270-1272.

Kang I-J, Kang Y-H, Chung C-K, Oh S-H, Lee J-W and Byun MW, 2005. Genotoxicological safety of high-dose irradiated porridges. Journal of the Korean Society of Food Science and Nutrition 34, 261-266.

Khan AA, Khan HM and Delincée H, 2003. "DNA Comet assay" - a validity assessment for the identification of radiation treatment of meats and seafood. European Food Research and Technology 216, 88-92.

Khan AA, Khan HM and Delincée H, 2005a. DNA Comet Assay - A rapid screening method for detection of irradiated cereals and tree nuts. Food Control 16, 141-146.

Khan AA, Khan HM and Wasim MA, 2005b. Identification of radiation treatment of foods using novel technique of "DNA Comet assay". Journal of the Chemical Society of Pakistan 27, 65-68.

Kilcast D. 1994. Effect of irradiation on vitamins. Food Chemistry 49, 157-164.Kim M-J, Lee J-W, Seo J-H. Song H-P, Yook H-S, Choi J-M and Byun M-W, 2003. Safety Evaluation on

Mutagenicity of White Layer Cake Containing Gamma-Irradiated Egg White. Journal of the Korean Society of Food Science and Nutrition 32, 1172-1173.

Kim M-J, Lee J-W, Seo J-H, Song H-P, Yook H-S, Choi J-M and Byun M-W, 2003. Safety Evaluation on Mutagenicity of White Layer Cake Containing Gamma-Irradiated Egg White. J. Korean Society of Food Science and Nutrition 32, 1172-1175.

Kim JH, Seo HY and Kim KS, 2004. Analysis of radiolytic products of lipid in irradiated dried squids (*Todarodes pacificus*). Journal of Food Protection 67, 1731-1735.

Kitis G, Cruz Zaragoza E and Furetta C, 2005. Thermoluminescence properties of Chile Guajillo (paprika) Mexicano. Applied Radiation and Isotopes 63, 247-254.

Kizil R, Irudayaraj J and Seetharaman K, 2002. Characterization of irradiated starches by using FT-Raman and FTIR spectroscopy. Journal of Agricultural and Food Chemistry 50, 3912-3918.

Knoll N, Weise A, Claussen U, Sendt W, Marian B, Glei M and Pool-Zobel BL, 2006. 2-Dodecylcyclobutanone, a radiolytic product of palmitic acid, is genotoxic in primary human colon cells and in cells from preneoplastic lesions. Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis 594, 10-19.

Korkmaz M and Polat M, 2004. An electron spin resonance study of radicals induced by radiation in oat (*Avena sativa L.*). International Journal of Food Science and Technology, 39, 975-983.

Kume T, Furuta M, Todoriki S, Uenoyama N and Kobayashi Y, 2009. Status of food irradiation in the world. Radiation Physics and Chemistry 78, 222-226.

Lee EJ, Love J and Ahn DU, 2003. Effect of antioxidants on consumer acceptance of irradiated turkey meat. Journal of Food Science 68, 1659-1663.

Lee J, Kausar T and Kwon JH, 2008. Characteristic Hydrocarbons and 2-Alkylcyclobutanones for Detecting gamma-Irradiated Sesame Seeds after Steaming, Roasting, and Oil Extraction. Journal of Agricultural and Food Chemistry 56, 10391-10395.

Lee JI, Kang S, Ahn DU and Lee M, 2001a. Formation of cholesterol oxides in irradiated raw and cooked chicken meat during storage. Poultry Science, 80, 105-108.

Lee JW, Kim JH, Yook HS, Kang KO, Lee SY, Hwang HJ and Byun MW, 2001b. Effects of gamma radiation on the allergenic and antigenic properties of milk proteins. Journal of Food Protection 64, 272-276.

Lee JW, Seo JH, Kim JH, Lee SY, Kim KS and Byun MW, 2005. Changes of the antigenic and allergenic properties of a hen's egg albumin in a cake with gamma-irradiated egg white. Radiation Physics and Chemistry 72, 645-650.

Letellie.Pr and Nawar WW, 1972. 2-alkylcyclobutanones from radiolysis of triglycerides. Lipids 7, 75-76.

Leth T, Hansen HB and Boisen F, 2006. Comparison of three methods for detection of herbal food supplement irradiation. European Food Research and Technology 223, 39-43.

Mangiacotti M, Chiaravalle AE, Marchesani G, De Sio A, Boniglia C, Bortolin E and Onori S, 2009. Detection of irradiated chestnuts: preliminary study using three analytical techniques. Radiation Physics and Chemistry 78, 695-698.

Maratea KA, Hooser SB, Ramos-Vara JÁ, 2006. Degenerative myelopathy and vitamin A deficiency in a young black-maned lion (Panthera leo). Journal of Veterinary Diagnostic Investigation 18, 608–611.

Marchioni E, Horvatovich P, Charon H and Kuntz F, 2005a. Detection of irradiated ingredients included in low quantity in non-irradiated food matrix. 1. Extraction and ESR analysis of bones from mechanically recovered poultry meat. Journal of Agricultural and Food Chemistry 53, 3769-3773.

Marchioni E, Horvatovich P, Charon H and Kuntz F, 2005b. Detection of irradiated ingredients included in low quantity in non-irradiated food matrix. 2. ESR analysis of mechanically recovered poultry meat and TL analysis of spices. Journal of Agricultural and Food Chemistry 53, 3774-3778.

Marchioni E, Horvatovich P, Ndiaye B, Miesch M and Hasselmann C, 2002. Detection of low amount of irradiated ingredients in non-irradiated precooked meals. Radiation Physics and Chemistry 63, 447-450.

Marin-Huachaca NS, Mancini J, Delincée H and Villavicencio ALCH, 2004. Identification of gamma-irradiated papaya, melon and watermelon. Radiation Physics and Chemistry 71, 193-196.

Marin-Huachaca N, Delincée H, Mancini J and Villavicencio ALCH, 2005. Use of the DNA Comet Assay to detect beef meat treated by ionizing radiation. Meat Science 71, 446-450.

McNeal TP, Nyman PJ, Diachenko GW, Hollifield HC, 1993. Survey of Benzene in Foods by Using Headspace Concentration Techniques and Capillary Gas-Chromatography. Journal of AOAC International 76, 1213-1219.

Miyahara M, Nagasawa T, Akiyama S, Kobayashi Y, Mashimizu T and Maitani T, 2004. ESR method for the detection of irradiated unboned meats and seafood. Journal of Health Science 50, 542-544.

Morehouse KM and Ku Y, 1993. Identification of irradiated foods by monitoring radiolytically produced hydrocarbons. Radiation Physics and Chemistry 42, 359-362.

Müller H and Diehl JF. 1996. Effect of inonizing radiation on floates in food. Lebensmittel Wiss u-Technology 29, 187-190.

Nam KC, Du M, Jo C, Ahn DU, 2001. Cholesterol oxidation products in irradiated raw meat with different packaging and storage time. Meat Science 58, 431-435.

Nam KC and Ahn DU, 2003. Double-packaging is effective in reducing lipid oxidation and off-odor volatiles of irradiated raw Turkey meat. Poultry Science 82, 1468-1474.

Nawar WW, Shu ZR and Yoo YJ, 1990. Radiolytic products of lipids as markers for the detection of irradiated meats. Meat, poultry and game, pp. 13-24.

Nazzaro F, Fratianni F, Picariello G, Coppola R, Reale A and Di Luccia A, 2007. Evaluation of gamma rays influence on some biochemical and microbiological aspects in black truffles. Food Chemistry 103, 344-354.

O'Bryan CA, Crandall PG, Ricke SC and Olson DG, 2008. Impact of irradiation on the safety and quality of poultry and meat products: A review. Critical Reviews in Food Science and Nutrition 48, 442-457.

Obana H, Furuta M and Tanaka Y, 2006. Detection of 2-alkylcyclobutanones in irradiated meat, poultry and egg after cooking. Journal of Health Science 52, 375-382.

Obana H, Furuta M and Tanaka Y, 2007. Detection of irradiated meat, fish and their products by measuring 2-alkylcyclobutanones levels after frozen storage. Journal of the Food Hygienic Society of Japan, 48, 203-206.

Oh KN, Lee SY, Lee HJ, Kim KE and Yang JS, 2003. Screening of gamma irradiated spices in Korea by using a microbiological method (DEFT/APC). Food Control 14, 489-494.

Palmer AC, Cavanagh JB, 1995. Encephalomyelopathy in young cats. The Journal of Small Animal Practice 36,57–64.

Palmer AC, Callinan JJ, Guerin LA, Sheahan BJ, Stronach N, Franklin RJ, 2001. Progressive encephalomyelopathy and cerebellar degeneration in 10 captive-bred cheetahs. The Veterinary Record, 149, 49-54.

Raffi JJ, Agnel JPL, Frejaville CM and Saintlebe LR, 1981a. Radio-induced products in maize starch - glyceraldehyde, dihydroxyacetone, and 2-hydroxymalonaldehyde. Journal of Agricultural and Food Chemistry 29, 548-550.

Raffi JJ, Agnel JPL, Thiery CJ, Frejaville CM and Saintlebe LR, 1981b. Study of gamma-irradiated starches derived from different foodstuffs - a way for extrapolating wholesomeness data. Journal of Agricultural and Food Chemistry 29, 1227-1232.

Raul F, Gosse F, Delincée H, Hartwig A, Marchioni E, Miesch M, Werner D and Burnouf D, 2002. Food-borne radiolytic compounds (2-alkylcyclobutanones) may promote experimental colon carcinogenesis. Nutrition and Cancer 44, 188-191.

Read MS, Trabosh HM, Worth WS, Kraybill HF and Witt NF, 1959. Short-term rat-feeding studies with gamma-irradiated food products .2. Beef and pork stored at elevated temperature. Toxicology and Applied Pharmacology 1, 417-425.

Read MS, Witt NF, Kraybill HF, Isaac GJ, Worth WS and Thompson SW, 1961. Successive generation rat feeding studies with a composite diet of gamma-irradiated foods. Toxicology and Applied Pharmacology 3, 153-173.

Sanderson DCW, Carmichael LA and Fisk S, 2003a. Photostimulated luminescence detection of irradiated herbs, spices, and seasonings: International interlaboratory trial. Journal of Aoac International 86, 990-997.

Sanderson DCW, Carmichael LA and Fisk S, 2003b. Photostimulated luminescence detection of irradiated shellfish: International interlaboratory trial. Journal of Aoac International 86, 983-989.

Sanderson DCW, Carmichael LA and Fisk S, 2003c. Thermoluminescence detection of irradiated fruits and vegetables: International interlaboratory trial. Journal of Aoac International 86, 971-975.

Savage GP, Dutta PC and Rodriguez-Estrada MT, 2002. Cholesterol oxides: their occurrence and methods to prevent their generation in foods. Asia Pacific Journal of Clinical Nutrition 11, 72-78.

SCF, 1986. Food Science and Tecniques. Reports of the Scientific Committee for Food (Eighteenth Series).

SCF, 1992. Food Science and Tecniques. Reports of the Scientific Committee for Food (Thirty-second Series).

SCF, 1998. Opinion of the Scientific Committee on Food on the irradiation of eight foodstuffs .

SCF, 2002. Statement of the Scientific Committee on Food on a Report on 2-alkylcyclobutanones.

SCF, 2003. Revision of the opinion of the Scientific Committee on Food on the irradiation of food.

Sin DWM, Wong YC, Yao MWY and Marchioni E, 2005. Identification and stability study of irradiated chicken, pork, beef, lamb, fish and mollusk shells by electron paramagnetic resonance (EPR) spectroscopy. European Food Research and Technology 221, 684-691.

Sin DWM, Wong YC and Yao MWY, 2006. Application of pentafluorophenyl hydrazine on detecting 2-dodecylcyclobutanone and 2-tetradecylcyclobutanone in irradiated chicken, pork and mangoes by gas chromatography-mass spectrometry. European Food Research and Technology, 222, 674-680.

Sjoberg AM, Manninen M, Harmala P and Pinnioja S, 1990. Methods for detection of irradiation of spices. Zeitschrift fur Lebensmittel-Untersuchung und -Forschung 190, 99-103.

Sokhey AS and Chinnaswamy R, 1993. Chemical and molecular-properties of irradiated starch extrudates. Cereal Chemistry 70, 260-268.

Sokhey AS, Hanna MA, Wootton M and Gallant DJ, 1993. Properties of irradiated starches. Food Structure 12, 397-410.

Sommers CH, 2003. 2-Dodecylcyclobutanone does not induce mutations in the Escherichia coli tryptophan reverse mutation assay. Journal of Agricultural and Food Chemistry 51, 6367-6370.

Sommers CH, 2006. Induction of micronuclei in human TK6 lymphoblasts by 2-dodecylcyclobutanone, a unique radiolytic product of palmitic acid. Journal of Food Science 71, C281-C284.

Sommers CH and Mackay WJ, 2005. DNA damage-inducible gene expression and formation of 5-fluorouracil-resistant mutants in Escherichia coli exposed to 2-dodecylcyclobutanone. Journal of Food Science 70, C254-C257.

Sommers CH and Schiestl RH, 2004. 2-dodecylcyclobutanone does not induce mutations in the Salmonella mutagenicity test or intrachromosomal recombination in Saccharomyces cerevisiae. Journal of Food Protection 67, 1293-1298.

Stefanova R, Vasilev NV and Spassov SL, 2010. Irradiation of food, current legislation framework, and detection of irradiated foods. Food Analytical Methods 3, 225-252.

Stevenson MH, 1994. Identification of irradiated foods. Food Technology 48, 141-144.

Stewart EM, Moore S, Graham WD, McRoberts WC and Hamilton JTG, 2000. 2-Alkylcyclobutanones as markers for the detection of irradiated mango, papaya, Camembert cheese and salmon meat. Journal of the Science of Food and Agriculture 80, 121-130.

Stewart EM, McRoberts WC, Hamilton JTG and Graham WD, 2001. Isolation of lipid and 2-alkylcyclobutanones from irradiated foods by supercritical fluid extraction. Journal of Aoac International 84, 976-986.

Stewart EM, 2009a. Effect of gamma irradiation on the quality of ready meals and their meat components. In: Irradiation to Ensure the Safety and Quality of Prepared Meals. Results of the Coordinated Research Project organised by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture (2002-2006). IAEA, Vienna, p 313-342.

Stewart EM, 2009b. Food Irradiation. In: Process-Induced Food Toxicants – Occurrence, Formation, Mitigation and Health Risks (Stadler, R.H. and Lineback, D.R., Eds.), Chapter 4.2, pp. 387-412.

Su M, Venkatachalam M, Teuber SS, Roux KH and Sathe SK, 2004. Impact of gamma-irradiation and thermal processing on the antigenicity of almond, cashew nut and walnut proteins. Journal of the Science of Food and Agriculture 84, 1119-1125.

Sung-Kee J, Yeon-Ho H, Hae-Ran P, Heon O and Myung-Woo B, 2001. Genotoxicological safety of hot water extracts of the gamma-irradiated Glycyrrhizae radix, Aurantii nobilis pericarpium and Bupleuri radix in. Journal of the Korean Society of Food Science and Nutrition, 30, 1237-1245.

Tai CY, Chen YC and Chen BH, 1999. Analysis, formation and inhibition of cholesterol oxidation products in foods: An overview (Part I). Journal of Food and Drug Analysis 7, 243-257.

Terry AJ and McColl NP (National Radiological Protection Board), 1992. Radiological consequences of food irradiation. R247, 16.

Tewfik I, 2008a. Extraction and identification of cyclobutanones from irradiated cheese employing a rapid direct solvent extraction method. International Journal of Food Sciences and Nutrition 59, 590-598.

Tewfik I, 2008b. Inter-laboratory trial to validate the direct solvent extraction method for the identification of 2-dodecylcyclobutanone in irradiated chicken and whole liquid egg. Food Science and Technology International 14, 277-283.

Tewfik I and Tewfik S, 2008. Would it still be possible to identify irradiated lipid-containing foods towards the end of their shelf-life? Food Science and Technology International, 14, 519-524.

Thayer DW, 1990. Food irradiation - benefits and concerns. Journal of Food Quality 13, 147-169.

Underdal B, Nordal J, Lunde G and Eggum B, 1976. The effect of ionising radiation on the nutritional value of mackerel. Lebensmittel Wiss. Technology 9, 72-74.

Variyar PS, Chatterjee S, Sajilata MG, Singhal RS and Sharma A, 2008. Natural Existence of 2-Alkylcyclobutanones. Journal of Agricultural and Food Chemistry 56, 11817-11823.

Villavicencio ALCH, Araujo MM, Baldasso JG, Aquino S, Konietzny U and Greiner R, 2004a. Irradiation influence on the detection of genetic-modified soybeans. Radiation Physics and Chemistry, 71, 491-494.

Villavicencio ALCH, Araujo MM, Marin-Huachaca NS, Mancini J and Delincée H, 2004b. Identification of irradiated refrigerated poultry with the DNA Comet assay. Radiation Physics and Chemistry 71, 189-191.

Watabe T and Yamada N, 1975. Biotransformation of 1-hexadecane to carcinogenic 1,2-epoxyhexadecane by hepatic microsomes. Biochemical Pharmacology 24, 1051-1053.

WHO (World Health Organization), 1994. Safety and nutritional adequacy of irradiated food. World Health Organization, Geneva.

WHO (World Health Organization), 1999. High-dose irradiation: wholesomeness of food irradiated with doses above 10 kGy. Report of a Joint FAO/IAEA/WHO Expert, Committee. Geneva, World Health Organization, 1999 (WHO Technical Report, Series, No. 890).

Yook H-S, Cha B-S, Kim D-H, Lee J-W and Byun M-W, 2004. Genotoxicological safety of gamma-irradiated salted and fermented anchovy sauce. Journal of Korean Society of Food Science and Nutrition 33, 1192-1200.

Yook HS, Byun MW, Song HP, Lee JW, Kim KS, Kim KH, Lee HJ and Kim DH, 2005. Assurance on the genotoxicological safety of fermented vegetables pasteurizied by gamma irradiation. Food Science and Biotechnology 14, 137-142.

Young-Beob Y and Sung-Kee J, 2000. Evaluation on the safety of gamma-irradiated Angelica gigas Nakai: stability of active components and safety in genotoxicity test. Journal of the Korean Society of Food Science and Nutrition, 29, 300-306.

Yu Y-B, Jeong I-Y, Park HR, Oh H, Jung U and Jo S-K, 2004. Toxicological safety and stability of the components of an irradiated Korean medicinal herb, Paeoniae Radix. Radiation Physics and Chemistry 71, 115-119.

Zanardi E, Battaglia A, Ghidini S, Conter M, Badiani A and Ianieri A, 2007. Evaluation of 2-alkylcyclobutanones in irradiated cured pork products during vacuum-packed storage. Journal of Agricultural and Food Chemistry 55, 4264-4270.

Zhang X, Julien-David D, Miesch M, Raul F, Geoffroy P, Aoude-Werner D, Ennahar S and Marchioni E, 2006. Quantitative analysis of beta-sitosterol oxides induced in vegetable oils by natural sunlight, artificially generated light, and irradiation. Journal of Agricultural and Food Chemistry 54, 5410-5415.

Zhenxing L, Hong L, Limin C and Jamil K, 2007. The influence of gamma irradiation on the allergenicity of shrimp (Penaeus vannamei). Journal of Food Engineering 79, 945-949.

Zegota H, 1988. Suitability of Dukat strawberries for studying effects on shelf life of irradiation combined with cold storage. Zeitschrift fur Lebensmittel-Untersuchung und -Forschung 187, 111-114.

Zhu MJ, Mendonca A, Ismail HA, Du M, Lee EJ and Ahn DU, 2005. Impact of antimicrobial ingredients and irradiation on the survival of Listeria monocytogenes and the quality of ready-to-eat turkey ham. Poultry Science 84, 613-620.