SCIENTIFIC OPINION

Scientific Opinion on the efficacy and microbiological safety of irradiation of food[1]

EFSA Panel on Biological Hazards (BIOHAZ)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Opinion considers and discusses efficacy of food irradiation as the ability of irradiation to reduce food-borne pathogens in foods and microbiological safety of food irradiation as the contribution of irradiation to reduce the risks to human health from food-borne pathogens. The chemical safety and nutritional aspects of irradiation are addressed in a separate Opinion by the EFSA Panel on Food contact materials, enzymes, flavourings and processing aids. Potential microbiological risks linked to food irradiation are reviewed and the Opinion confirms that there are no microbiological risks for the consumer linked to the use of food irradiation and its consequences on the food microflora. The Opinion recommends that food irradiation should be based on risk assessment and on the desired risk reduction rather than on predefined food classes/commodities and doses as proposed in the past. In addition, with respect to efficacy and microbiological safety, it is recommended that upper dose limits for pathogen reduction should not be specified, since other constraints, such as undesirable chemical changes, will limit the doses applied. Irradiation should be considered as one of several approaches to reducing pathogens in food and thus helping to ensure protection of consumers' health. It is also recommended that food irradiation should only be used in conjunction with an integrated food safety management program and the Opinion concludes that when this includes GAP, GHP, GMP and HACCP, and depending on the dose applied, food irradiation can contribute to improved consumer safety by reducing food-borne pathogens.

KEY WORDS

Food irradiation, efficacy, microbiological safety, food-borne pathogens.

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SUMMARY

Following a request from the European Commission, the Panel on Biological Hazards (BIOHAZ Panel) was asked to deliver a scientific Opinion on the irradiation of food (efficacy and microbiological safety). Using the previous Opinions of the Scientific Committee on Food (SCF) on irradiation of food, EFSA was asked to establish whether the food classes/commodities and doses specified in those Opinions are still up-to-date to ensure consumer safety.

Within this Opinion the BIOHAZ Panel considers and discusses efficacy of food irradiation as the ability of irradiation to reduce food-borne pathogens in foods and microbiological safety of food irradiation as the contribution of irradiation to reduce the risks to human health from food-borne pathogens. The chemical safety and nutritional aspects of irradiation are not considered in this Opinion as they are addressed in a separate Opinion by the EFSA Panel on Food contact materials, enzymes, flavourings and processing aids (CEF Panel).

This Opinion also evaluates, for each food class/commodity requested, any recent change in biological hazards and food processing, preparation or consumption patterns which could have modified the risks for consumers and whether irradiation can be used in combination with other treatments and how its efficacy can be compared to other methods.

The Opinion briefly presents the technologies available for the irradiation of food. Different technologies are currently available, having to a large extent the same effect on microorganisms. While gamma-rays are produced from a radioactive source, e-beams and X-rays are produced by specific equipment converting other energy sources, without the involvement of any radioactive substance.

Potential microbiological risks linked to food irradiation are reviewed and the Opinion confirms that there are no microbiological risks for the consumer linked to the use of food irradiation and its consequences on the food microflora.

An update of the scientific knowledge on the irradiation of the food classes/commodities to be considered is provided, including a review of the efficacy of irradiation. However, it is stated that these food classes/commodities do not represent, at this time, a systematic classification of foods with respect to irradiation practices and that therefore the categorisation of foods to assess the efficacy of irradiation to inactivate pathogens is not necessary. In addition, the food marketing practices and consumption patterns have changed in recent years and the previous classification did not identify all foods representing a potential high risk for consumers (e.g. some ready-to-eat foods).

The Opinion concludes that the irradiation dose needed to inactivate food-borne pathogens depends on the targeted pathogen, on the reduction required and on the physical state of the food, regardless of the food classes as previously proposed. It is also recommended that food irradiation should be based on risk assessment and on the desired risk reduction rather than on predefined food classes/commodities and doses. Finally, with respect to efficacy and microbiological safety, it is recommended that upper dose limits for pathogen reduction should not be specified. It is highlighted that other constraints, such as undesirable chemical changes, will limit the doses applied.

It is recommended to consider irradiation as one of several approaches to reducing pathogens in food and thus helping to ensure protection of consumers' health. The Opinion also recommends that food irradiation should only be used in conjunction with an integrated food safety management program. When this includes Good Agricultural, Hygienic and Manufacturing Practices (GAP, GHP, GMP) and HACCP, and depending on the dose applied, food irradiation can contribute to improved consumer safety by reducing food-borne pathogens in all the food categories and food commodities addressed by the present Opinion.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The Scientific Committee on Food (SCF) expressed Opinions on irradiated foods in 1986, 1992 and 1998 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, 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 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. 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.

It was also clarified that both the CEF Panel, that will provide a scientific Opinion on the chemical safety of the treatments, and the BIOHAZ Panel, that will address the efficacy and the microbiological safety of the treatments, will be involved. A coordinated approach will be followed by the CEF and the BIOHAZ Panels when developing their respective scientific Opinions.

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

ASSESSMENT

1. Preamble

1.1. Understanding of the mandate and scope of the Opinion

Food irradiation has been proposed for a large range of purposes, but the Opinion from the EFSA Panel on Biological Hazards (BIOHAZ Panel) will only consider efficacy of food irradiation as the ability of irradiation to reduce food-borne pathogens in foods. Within this Opinion, microbiological safety of food irradiation will be understood as the contribution of irradiation to reduce the risks to human health from food-borne pathogens. The BIOHAZ Panel will also consider 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, etc. The chemical safety and nutritional aspects of irradiation will not be considered in this Opinion as it will be addressed in a separate Opinion by the EFSA Panel on Food contact materials, enzymes, flavourings and processing aids (CEF Panel).

The Opinion will follow the general food classes or specific food commodities and doses proposed in the terms of reference. However, for several food classes/commodities, in the past irradiation doses were defined for purposes other than consumer safety (e.g. sprouting inhibition, disinfestation, shelf-life extension). The efficacy of irradiation to achieve these purposes will not be discussed in this Opinion. Nevertheless, the efficacy of the doses, recommended in the previous Reports/Opinions of the Scientific Committee of Food (SCF) (SCF, 1986, 1992, 1998) for these food classes/commodities will be discussed in respect to the reduction of food-borne pathogens.

The present Opinion will also evaluate, for each food class/commodity, any change, since the previous SCF Reports/Opinions, in biological hazards and food processing, preparation or consumption patterns, which could have modified the risks for consumers.

In several food classes or commodities, the dose applied is limited by the negative impact of too high irradiation doses on food sensory and/or technological quality. These limitations depend on many factors, such as the quality criteria considered, the usage of the food, the other technologies associated to the irradiation treatment, and the specific nature of the food (e.g. fruit cultivars, etc.). The impact of irradiation on food quality is not within the purposes of this Opinion and will only be discussed in the extent it could limit the efficacy of food irradiation to inactivate food-borne pathogens.

Whether irradiation can be used in combination with other treatments and how its efficacy can be compared to other methods will also be considered.

The rationale of considering each food class/commodity while assessing the efficacy and microbiological safety of irradiation will be discussed. In particular, the effect of irradiation on food-borne pathogens depends on many factors: some are related to the food class/commodity (e.g. the food substrate), while many others are more general (e.g. the dose applied, the intrinsic resistance of the pathogen, the physical conditions, etc.). Similarly, the risks linked to food irradiation with regards to food-borne pathogens (e.g. development of resistance, etc.) do not depend on the food class/commodity. Therefore the general knowledge on efficacy and risks linked to food irradiation will also be updated in the present Opinion.

1.2. Previous SCF Reports/Opinions

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 technological purposes, rather than for microbiological safety.

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)	Dose (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): assessed by SCF (1986)
(b): assessed by SCF (1992)
(c): assessed by SCF (1998)
*: 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.

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 the 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, technological 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 so also as a follow up of the conclusions of a Joint FAO/IAEA/WHO Study Group on high-dose irradiation, which concluded that the data on radiation chemistry, toxicology, microbiology and nutritional properties of foods treated with radiation at doses above 10kGy were adequate. The study group further concluded that food irradiated to any dose appropriate to achieve the intended technological 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*".

1.3. Previous FAO/WHO assessments

The FAO/WHO/IAEA Study Group on "High-Dose Irradiation: Wholesomeness of Food Irradiated with Doses above 10 kGy", met from 1997 (see Appendix B for more details), to assess the safety and nutritional adequacy of food irradiated to doses above 10 kGy (WHO, 1999). The Study Group was formed in response to the technological need for average doses higher than 10 kGy to ensure that food items, particularly meat and poultry, are rendered consistently free of pathogens. The Study Group examined other technological objectives of high-dose irradiation, including the decontamination of low-moisture products, such as spices, herbs, and dried vegetables, the preparation of sterilized meals or meal components for hospitalized patients, and the production of shelf-stable hygienic products that reduce the need for refrigeration and frozen storage and can thus facilitate safe food distribution under tropical and subtropical conditions.

The experts considered evidence that foods of similar composition show similar microbiological responses when similarly irradiated, thus supporting the validity of granting broadly-based generic approvals of high-dose irradiated foods.

The effects of irradiation on microorganisms and the factors influencing their radiation resistance were reviewed. Studies evaluated covered vegetative bacterial cells, animal parasites, yeasts, mould propagules, bacterial spores, viruses, and preformed microbial toxins. On the basis of this exhaustive review, the report concluded that high-dose irradiation is no different from thermal processing in producing shelf-stable, microbiologically safe foods.

The Study Group addressed the important role packaging plays in facilitating irradiation processing, in protecting irradiated food from recontamination, and in maintaining the quality of the food. The Study Group report also considered the processing and environmental conditions and control procedures essential for ensuring that a food product is sterilized within the targeted dose range.

2. Introduction

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, under EU legislation, Good Hygiene Practice (GHP) and HACCP principles must be applied, with a few exceptions (e.g. HACCP for primary production), throughout the entire food chain (as laid down by Regulation (EC) No 852/2004[4]).

Measures to control biological hazards in the food chain are primarily preventive measures, to avoid contamination with food-borne pathogens. Methods relying on destruction of micro-organisms that could have contaminated the food are only additional control measures, as illustrated by past Opinions of the Scientific Committee on Veterinary Measures relating to Public Health (SCVPH) (SCVPH, 1998, 2003), which stated that antimicrobial treatments should be considered as supplementary means of reducing microbial loads of foods of animal origin and should be part of an integrated program throughout the food chain. According to Directive 1999/2/EC[5], irradiation of food can be used "*to reduce the incidence of foodborne disease by destroying pathogenic organisms*" and one of the preconditions to its authorisation is that "*it is not used as a substitute for hygiene and health practices or for good manufacturing or agricultural practice*".

The extent of destruction of pathogenic microorganisms in foods needed to reduce the incidence of food-borne diseases depends on many factors and cannot be fixed *a priori*.

Methods to inactivate micro-organisms present on, or in, foods usually cause some modifications in the characteristics of the food which may limit their application. However, food irradiation has been investigated as a method which could kill micro-organisms while causing fewer changes in food characteristics compared to heat or chemical treatments.

2.1. Food irradiation technologies

Different technologies are currently available and used for the irradiation of food. As foreseen by the Codex General Standard for Irradiated Foods[6], such technologies are primarily based on the use of three different kinds of ionising radiation:

- gamma rays (γ-rays) from the radionuclides cobalt-60 (Co-60) or cesium-137 (Cs-137);
- X-rays generated from machine sources operated at or below an energy level of 5 MeV;
- electrons (e-beams) generated from machine sources operated at or below an energy level of 10 MeV.

While the first type (γ-rays) are produced from a radioactive source, the other two (X-rays and e-beams) are produced by specific equipment converting other energy sources, such as electric current, without the involvement of any radioactive substance.

None of these kinds of ionising radiation, when used for food irradiation purposes at the doses established by Codex Standard and EU legislation, have energy levels sufficient to induce radioactivity in the irradiated food.

[4] 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, 30.4.2004, p-1-54, as last amended.
[5] Directive 1999/2/EC of the European Parliament and of the Council on the approximation of the laws of Member States concerning foods and food ingredients treated with ionising radiation. OJ L 66, 13.3.1999, p. 16–23, as last amended.
[6] CODEX STAN 106-1983, REV.1-2003.

Even if the ionising radiation used is different, the different ionising radiations have the same effects on microorganisms, although some studies found e-beams slightly more efficient than gamma rays for the inactivation of bacteria in meat (Lopez-Gonzalez et al., 1999; Miyahara and Miyahara, 2002; Rajkowski et al., 2006) and one study reported an efficacy of X-rays to inactivate *E. coli* O157 in lettuce higher than usually reported with other ionising radiations (Jeong et al., 2010).

2.1.1. Gamma-rays

γ-rays are given off by certain radioactive substances when decaying. As mentioned above, the radionuclides used for the irradiation of food are represented by Co-60 and Cs-137. The former is intentionally obtained by exposing Co-59 to neutrons in a nuclear reactor, while the latter is recovered from nuclear reactor operations. Such radionuclides are characterized by a half-life of 5.26 years (Co-60) and 30.1 years (Cs-137) and emit γ-rays continuously. Among them, Co-60 is more commonly used for food irradiation purposes, while Cs-137 is less used because of its limited availability.

The absorbed dose in materials exposed to irradiation with γ-rays decreases exponentially with increasing depth and the penetration is inversely proportional to the average density of the material irradiated (Cleland, 2006).

For consideration of penetrability of ionising radiation, water serves as a sufficiently good model for what occurs with high moisture foods. The penetration of γ-rays, electron beams and X-rays into matter occurs in different ways. In the case of γ-rays, the half-value thickness for water is 10.8 cm for Co-60 gamma photons, and 8.2 cm for Cs-137 (Urbain, 1986).

2.1.2. Electron beams

As mentioned above, e-beams are produced in a different way compared to γ-rays. E-beams consist of a stream of high energy electrons accelerated by specific equipment that converts electricity. Therefore, e-beams are not derived from radioactive substances and the producing equipment can be switched on or off depending on the need. Several methods are used to produce e-beams, including constant-potential accelerators, direct-current systems, microwave linear accelerators and radio-frequency accelerators (Cleland, 2006).

Compared to γ-rays, e-beams are characterized by a low penetrative capacity. They can only penetrate food up to a depth of a few centimeters, which can limit the type of food that can be processed. For example, e-beam irradiation is particularly useful for products like grain, which can be processed in thin layers (WHO, 1988), or surface contaminated products.

2.1.3. X-rays

X-rays are a further evolution of e-beams. Electrons are directed towards plates made of specific material (e.g. tantalum, tungsten, gold), generating a stream of X-rays coming out from the other side of the plate. The conversion efficiency from electrons to X-rays increases with the atomic number of the target material and with the electron energy. Increasing the energy also improves the x-ray penetration. In addition, X-rays are more concentrated in the forward direction compared to electrons (Cleland, 2006; Meissner et al., 2000). These characteristics make X-rays more suitable for the treatment of thicker packages and food commodities compared to e-beams and even to γ-rays. The half-value thickness for water and water-equivalent materials are 23 cm for 5.0 MeV X-rays (Urbain, 1986).

As in the case of e-beams, X-rays are not produced from radioactive substances and the equipment for their production can be simply switched on or off depending on the need.

2.2. Dosimetry aspects

The irradiation dose received by a food is not homogeneous because of the limits in penetration capacity of ionising radiations. This can reduce the overall efficacy of the treatment and the minimal dose received must be considered for implementation of food irradiation in practice. However this aspect will not be discussed in the present Opinion, which is focused on the relation between dose received and efficacy on food-borne pathogens. These aspects of dosimetry are discussed in the Opinion of the EFSA CEF Panel on Irradiation of food (chemical safety) (EFSA Panel on Food contact materials, enzymes, flavourings and processing aids (CEF), 2011).

2.3. Effects of irradiation on micro-organisms

The main reason for the use of food irradiation is the ability of ionising radiation to inactivate, to varying extents, populations of microorganisms, including pathogenic bacteria, parasites and viruses.

Inactivation of microorganisms is due to damage to critical elements of the cell, often the genetic material, which can be lethal or prevent multiplication of the cell. The interaction between the ionising radiation and the genetic material can be direct, with radiation causing breaks in one or both DNA strands. These lesions may be lethal by themselves or result in damage to the DNA strands, which, to a certain extent, may be also repaired by the cell (Zahradka et al., 2006). Recent work suggests that proteins could also be an important target of irradiation in prokaryotic cells (Daly et al., 2007).

Damage can also occur indirectly because of the interaction of radiation with molecules adjacent to genetic material, often represented by water. Irradiation of water results in the production of reactive molecules, such as hydroxyl radicals, hydrogen peroxide and hydrogen atoms, which then induce on the adjacent genetic material similar effects to those resulting directly by radiation (Ahn and Lee, 2006; Dickson, 2001; WHO, 1999).

Radiation may also cause a number of lethal or sub-lethal effects in other structures of the cells, such as membranes, enzymes and plasmids.

Dickson (2001) stresses the fact that it is difficult to separate the effects of genetic and nongenetic damage caused by irradiation and that one important aspect of this point is that the damage is random and not related to a specific genetic locus or cell component, which is a significant factor complicating the elucidation of the radiation resistance of bacteria.

The effect of radiation on microorganisms is also affected by the environmental conditions under which the microorganisms are irradiated. Such factors include temperature, water activity, pH, chemical composition of the food, gaseous environment etc., which are considered in Chapter 3 of this Opinion.

2.4. Main objectives and applications

The radiation dose used in food processing is determined by the type of food being processed and the desired effects. The main purposes of food irradiation and examples of recommended dose ranges for various purposes are listed in Table 2.

Applications up to 1 kGy, between 1 and 10 kGy, and higher than 10 kGy are referred to as low-dose, medium-dose, and high-dose irradiation, respectively.

Table 2: Main purposes of food irradiation and examples of recommended dose ranges (adapted and simplified from Wilkinson and Gould (1996)).

Purpose and effects	Dose range (kGy)*
Inhibition of sprouting of stored tubers, roots and bulbs	0.05-0.15
Prevention of post-harvest losses by destruction of insects in stored cereals, fresh and dried fruits, nuts, oilseeds and pulses, or phytosanitary (quarantine) treatment for insect pests infesting fresh fruits and vegetables	0.15-1
Delay of ripening of fruits	0.2-1
Shelf-life extension of fruit and vegetables, meat, poultry, fish and ready meals by reduction of micro-organisms that cause spoilage	0.5-3
Inactivation/destruction of various food-borne parasites	0.3-6
Prevention of food-borne illness by destruction of non-sporeforming pathogenic bacteria (e.g. *Salmonella*, *Campylobacter*, *Listeria*) in fresh or frozen foods	3-7
Shorten drying and cooking times of vegetables and fruits	3-10
Reduction in viable counts of microorganisms in spices and other dry ingredients to minimize contamination of food to which the ingredients are added	5-10
Production of microbiologically shelf-stable, vacuum-packaged meat, poultry and ready-to-eat meals by heat-inactivating of their tissue-enzymes and sterilizing them by irradiation in deep-frozen state	up to 50

*The maximum doses reported are intended for good irradiation practice and not for consumer safety purposes.

2.5. Use of irradiation in the EU and third countries

2.5.1. EU

There are two main pieces of legislation regulating the irradiation of food at EU level: Directive 1999/2/EC and Directive 1999/3/EC[7] (see Appendix A for a brief description of the two Directives). Twelve Member States (Belgium, Bulgaria, Czech Republic, Germany, Spain, France, Hungary, Italy, the Netherlands, Poland, Romania and the United Kingdom) have, within their territory, a total of twenty-three irradiation facilities approved[8]. Approvals are granted by the competent authorities in Member States, in accordance with the procedure established by Directive 1999/2/EC. Besides the irradiation of foodstuffs listed in Directive 1999/3/EC (so far only "dried aromatic herbs, spices and vegetable seasonings" at the maximum overall average absorbed radiation dose of 10 kGy), irradiation of a number of foodstuffs is also temporarily admitted in certain Member States until a new Directive establishes an updated Community list. Foodstuffs and doses temporarily admitted at Member States level have been published by the Commission[9].

The Commission gathers each year data concerning the commodities irradiated in Member States and publishes an annual report summarizing those data. According to the last available report (EC, 2009), in 2007 8,154 tonnes 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

[7] Directive 1999/3/EC of the European Parliament and of the Council 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.
[8] See http://ec.europa.eu/food/food/biosafety/irradiation/approved_facilities_en.pdf, accessed on 21 September 2010.
[9] List of Member States' authorisations of food and food ingredients which may be treated with ionising radiation. OJ C 283/02, 24.11.2009, p.5.

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 and 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 Commission's website (EC, 2002, 2004, 2006a, 2006b, 2007, 2008); from a minimum of around 14,300 (2004) to a maximum of around 19,700 (2002) tonnes of irradiated food was reported globally in the EU.

It should be noted that in the above mentioned reports only food irradiated for the purposes falling under the scope of Directive 1999/2/EC is included. Therefore, food irradiated and intended for patients requiring sterile diets under medical supervision as well as food exposed to ionising radiation generated by measuring or inspection devices is not included.

2.5.2. Third countries

Food irradiation is approved for use in over 60 countries worldwide 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 third 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 and Vietnam.

Recent updates in national legislation have facilitated the use of irradiation as a post-harvest treatment, especially for quarantine and for food safety purposes. For example, the US Food and Drug Administration 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[10].

Kume et al. (2009) published a study outlining the state 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 amount of food irradiated worldwide in 2005 was almost 405,000 tonnes and classified the foods irradiated according to food category or purpose and geographic area, as briefly summarised in Table 3. China was the leading country in the use of food irradiation (146,000 tons) and three countries including USA (92,000 tons) and Ukraine (70,000 tons) made up three quarters of the total amount of food irradiated in the world in 2005. The authors reported that the main technology used was Co-60 irradiation, but that the use of electron beam and X-rays had recently increased.

[10] USFDA Regulation 21 CFR 179 26

Table 3: Volumes (tonnes) 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*

* Ukraine is considered by the Kume et al. (2009) as part of this region for the purpose of this table.

Currently, the International Atomic Energy Agency (IAEA) is responsible for updating and maintaining various irradiation databases[11] as resources for researchers, government officials and the general public, including:

- The *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.

- The *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.

Commission Decision 2002/840/EC[12] lays down a list of irradiation facilities located in third countries which are approved by the Community for irradiation of food to be imported into the EU. Ten facilities are included in that list, located in South Africa, Turkey, Switzerland, India, and Thailand.

Appendix B of this Opinion reports further information on the international background of the use of food irradiation.

[11] See http://nucleus.iaea.org

[12] Commission Decision 2002/840/EC adopting the list of approved facilities in third countries for the irradiation of foods. OJ L 287, 25.10.2002, p.40-41, as last amended.

3. Efficacy of food irradiation

3.1. D_{10} values

Microorganisms vary in their resistance to irradiation. The relative resistances of different species can be compared through the use of D_{10} values, where the D_{10} value is the dose required (in kGy) to achieve a 1-$\log_{10}$ reduction (or 90%) in viable numbers (see also Glossary). However, the response to irradiation may sometimes be nonlinear. An initial shoulder may be observed when plotting $\log_{10}$ numbers of survivors against dose. For example, when *Trichosporon cutaneum* was irradiated in phosphate buffered saline at 4°C, no inactivation was observed until the irradiation dose exceeded 4 kGy. Thereafter there was a linear inactivation with increasing dose (McCarthy and Damoglou, 1993). In contrast, more rapid decrease of the microbial population at the start of the treatment was also reported (Hvizdzak et al., 2010).

Viruses have relatively little nuclear material and are orders of magnitude smaller than bacteria and so, being smaller "targets", are relatively resistant to radiation. For example Hepatitis A virus in strawberries is reported to have a D_{10} value of 2.97 kGy (Bidawid et al., 2000). Also Rotavirus in oysters is reported to have a D_{10} value of 2.4 kGy (Mallett et al., 1991).

Yeasts, although not considered to be major food-borne pathogens, tend to be more resistant to radiation than vegetative bacteria. For example, *Cryptococcus albidus* was reported to have a D_{10} value of 2.5 kGy when chicken skin was irradiated at 10°C (Hughes, 1991).

With respect to fungi, D_{10} values of 0.32 and 0.44 kGy have been reported for *Penicillum expansum* and *Aspergillus ochraceus* in grain treated at 10°C (Oneill et al., 1991), while *Fusarium* spp. and *Alternaria* spp. are generally more resistant to radiation. However, like yeasts, it can be difficult to use the D_{10} concept unless it is applied to conidia rather than the mass of hyphae. Spores of *Fusarium* spp. and *Alternaria* spp. are multicellular and, therefore, if one cell escapes lethal damage, the spore may still have the ability to germinate. This may explain the shoulder observed in survivor curves of these relatively irradiation-resistant organisms (Casolari, 1988).

The most radiation-sensitive vegetative bacteria include *Pseudomonas* spp., *Vibrio* spp., *Campylobacter* spp., *Yersinia* spp., *Escherichia coli* O157:H7 and vegetative forms of *Bacillus cereus* (D_{10} values of 0.14-0.30 kGy, depending on the substrate and treatment conditions). Moderately resistant vegetative bacteria include *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus* and vegetative forms of *Clostridium perfringens* (D_{10} values 0.40-0.80 kGy) (SCF, 2003).

A number of naturally occurring extremely radiation-resistant bacteria have been isolated and originally identified as *Micrococcus* spp. viz. *M. radiodurans* (Anderson et al., 1956), *M. roseus* (Davis et al., 1963), *M. radiophilus* (Lewis, 1971) and *M. radioproteolyticus* (Kobataki et al., 1973). They were later assigned to a new genus, *Deinococcus* (Brooks and Murray, 1981). Another radiation-resistant bacterium isolated from pork (Grant and Patterson, 1989) was initially identified as a *Deinococcus* sp. and was later reclassified as *Hymenobacter actinosclerus* (Collins et al., 2000). These super-resistant microorganisms are not pathogenic, are sensitive to heat and are unlikely to cause a food safety or spoilage problem. The reason why such bacteria are resistant to irradiation is not completely understood, but it is likely to be linked to their unique ability to repair large numbers of double stranded DNA breaks (Moseley and Laser, 1965; Moseley, 1983; Zahradka et al., 2006) and to protect proteins from oxidation (Daly et al., 2007).

Bacterial spores are more resistant to irradiation than vegetative bacteria. *Clostridium botulinum* type A and B spores are particularly resistant with D_{10} values of over 3.4 kGy (ICMSF, 1996; Monk et al., 1995; Patterson and Loaharanu, 2000).

Parasites, including those that are snail-borne, fish-borne and crustacean-borne, liver flukes and *Trichinella* spp., are generally more sensitive to radiation than vegetative bacteria and doses below 1 kGy will prevent the most infective stage of parasites from infecting humans. However, it should be noted that radiation doses required for controlling infection of food-borne parasites may not necessarily kill them outright. For example, a minimum dose of 0.3 kGy will sterilize the most infective stage of the nematode *T. spiralis* without killing it (Gibbs et al., 1964). However, the infective stage of certain species can be more resistant to irradiation and it is reported that *Anisakis* spp. require higher doses for inactivation. For example, inactivation of *A. simplex* in salted herring required doses as high as 6-10 kGy (Van Mameren and Houwing, 1968). Similarly, another study found *A. simplex* larvae to be highly resistant to irradiation doses of 2 kGy or 10 kGy (Chai et al., 1991).

The activity of pre-formed bacterial toxins and mycotoxins is not affected by irradiation at the doses normally used in food applications.

Useful collections of D_{10} values of food-borne pathogens, e.g. bacteria, viruses, fungi and parasites, are available in the scientific literature (ICMSF, 1996; Monk et al., 1995).

3.2. Intrinsic and extrinsic factors affecting radiation D_{10} values and combinations of irradiation with other technologies

There are many published studies on the effect of irradiation on microorganisms and it is clear that D_{10} values are affected by a number of factors including temperature, water activity and chemical composition of the food (SCF, 1986, 2003).

In general, microbial resistance to irradiation is significantly less at ambient or chill temperatures than at freezing temperatures. In many cases the frozen state doubles the radiation resistance of vegetative bacteria (SCF, 1986). For example, the D_{10} value of *E. coli* O157 in red meat is 0.27 kGy at +5°C compared to 0.42 kGy at -5°C (Thayer and Boyd, 1993). Thus, irradiation treatment required for a 5-$\log_{10}$ reduction of this pathogen would be 1.35-2.1 kGy, depending on the temperature of the meat. The radiation resistance of spores is not significantly affected by freezing because the coat cortex and the forespore stage form a protective barrier against extracellular radicals.

D_{10} values are also higher in foods with a low water activity because the lack of water means that there are less $OH^{\bullet}$ radicals available to cause DNA damage. For this reason, higher doses are required to produce the desired microbial kill in dry foods such as spices.

The presence of proteins can exert a protective effect on microorganisms subjected to radiation treatment. This is because free radical scavengers, such as sulphydryl groups can mop up water and oxygen radicals. For example, it has been reported that *Salmonella* Enteritidis has a D_{10} value of 0.70 kGy and 0.49 kGy in low-fat and high-fat beef respectively. Assuming that low fat is associated with high protein, then it is possible that the radical scavenging properties of the protein in the low-fat-high-protein beef gave greater protection (Maxcy and Tiwari, 1973). Similarly, D_{10} values for *L. monocytogenes* in tortilla were 0.27 kGy, against 0.65 kGy in turkey meat (Sommers and Boyd, 2005).

For these reasons, it is important that information on the substrate and external factors such as temperature, water activity etc, are included when quoting D_{10} values.

Generally, any cells which survive irradiation are more sensitive to temperature, pH, nutrient deficiencies etc, compared to untreated cells. This is the rationale for combination treatments, allowing for the reduction in the required doses (Patterson and Loaharanu, 2000). For example, the combination of irradiation and heat is more effective because irradiation sensitises spores and

vegetative cells to heat inactivation, especially at radiation doses above 10 kGy. The combination of irradiation and modified atmosphere packaging (MAP) has potential as it could be possible to reduce the numbers of spoilage and pathogenic microorganisms by irradiation and suppress the growth of surviving microorganisms during storage, using MAP, without significantly affecting organoleptic quality. It is also reported that high hydrostatic pressure can sensitise *Clostridium* spores to subsequent irradiation (Crawford et al., 1996).

4. Possible microbiological concerns linked with the use of food irradiation

Regarding efficacy of irradiation and microbiological safety of irradiated food, a number of questions have been raised in the past, which have been considered already by the previous SCF reports (SCF, 1986, 2003), several Joint FAO/IAEA/WHO Expert Committee meetings on wholesomeness of irradiated food (WHO, 1966, 1970, 1977, 1981), a meeting of the Board of the International Committee on Food Microbiology & Hygiene of the International Union of Microbiological Societies (ICFMH, 1982) and various published extensive reviews (Diehl, 1995; Farkas, 1989; Gould, 1986; Ingram and Farkas, 1977; Maxcy, 1983; Satin, 1996).

Such questions can be summarised as follows:

- Could food irradiation be used to mask unhygienic practices?
- Could the selective effect of radiation on the microbial flora result in higher health risk, e.g. when harmless organisms are less radiation-resistant than certain pathogenic species, so that a more pathogenic population of microorganisms survives?
- Could mutations in the surviving population convert non pathogenic organisms to pathogenic organisms or less virulent strains to more virulent strains?
- Could irradiation stimulate toxin formation in toxin-producing bacteria or moulds?
- Could repeated sublethal treatments with radiation lead to increased radiation resistance?
- Could the diagnostic characteristics of microorganisms be changed as a result of irradiation so that the species or strains cannot be correctly identified or detected?
- Could the outer appearance of a spoiled food, possibly one containing a radiation resistant toxin, be improved by irradiation so that the consumer would not be warned by the usual danger signs, such as typical spoilage odour? Is irradiation only a “cosmetic” treatment?

Similar questions could be raised on any other established food preservation process.

A brief summary of answers to these questions is given below.

4.1. Integration in HACCP

As a pathogen reduction process, food irradiation is a possible critical control point of a HACCP risk management system (Smith and Pillai, 2004) and guidelines and codes of good irradiation practices are already established (listed e.g. in Diehl (1995)).

However, irradiation can also reduce levels of the background microflora of foods, including micro-organisms used as indicators of the hygiene of a process. Therefore, as for any other decontamination process, irradiation could, theoretically, be used to mask unhygienic processes or unhygienic harvest

conditions. For this reason only food produced under good agricultural, hygienic and manufacturing practices (GAP, GHP, GMP) shall be accepted for irradiation, as indicated by Directive 1999/2/EC.

4.2. Selective effects on the food microbial flora

Vegetative food pathogens are sensitive to radiation. The medium-dose irradiation processes reduce their populations by several $\log_{10}$ cycles, similar to non pathogenic microorganisms (see Chapter 3, Chapter 5 and Appendix C to the present Opinion). Therefore these pathogens will not have selective advantages compared to the surviving microbiota of irradiated foods. In addition, the possibility that reduction of the competitive microflora could facilitate growth of pathogens contaminating the food after irradiation was investigated. Dickson and Olson (2001) irradiated ground beef with 0, 2 and 4 kGy, thus reducing the spoilage microflora in the meat, and then inoculated it with *Salmonella* spp. or *Escherichia coli* O157:H7. The meat was stored at 4°, 15° or 25°C and bacterial growth was monitored over time. The study did not identify any significant difference in the bacterial growth in irradiated meat compared to non-irradiated one, suggesting that the absence of the spoilage microflora in ground beef does not provide a competitive advantage to the growth of these two bacteria. More recently Prendergast et al. (2009) examined the growth of pathogenic *E. coli* during storage at 10°C on the surface of irradiated and non-irradiated meat samples from different parts of beef carcasses. *E. coli* O157:H7 did not behave similarly on the various types of meat samples, but globally, it did not grow or survive better on irradiated samples.

Although bacterial spores are more resistant to radiation than vegetative bacteria, radiation technologies of processing perishable foods with non-sterilizing doses are combined e.g. with low storage temperature, making the food safe from spore-forming pathogens. Non-sterilizing radiation treatment is by no means unique with regard to selective effect on the microbiota. Similar precautions are needed with other non-sterilizing processes of perishable foods.

4.3. Effects on mutations

Mutation in bacteria and other organisms is a well known phenomenon and selection of spontaneous mutants or production of mutant strains by various mutagenic agents are practised for scientific and industrial purposes. Ionising radiation, in principle, is also able to induce mutations, just as a number of physical or chemical antimicrobial agents, e.g. ultraviolet radiation (also present in sunlight), food preservatives, drying or heat (Gould, 1986).

The SCF has also considered this question and concluded that "*there is some evidence that the pathogenicity of infectious organisms is diminished by irradiation. Moreover, should irradiation-induced enhanced infectivity be a problem, this would have become apparent from the many wholesomeness studies on irradiated foods carried out so far*" (SCF, 1986). There is no information to date that contradicts this statement. In recent experiments (Lim et al., 2007) on transcriptional changes of *S.* Typhimurium and *Vibrio* spp., the expression of the virulence genes in the fraction of *Salmonella* surviving gamma-radiation was reduced, and expression of toxin genes of *Vibrio* surviving irradiation did not increase, compared with non-irradiated counterparts.

There is no evidence either to indicate that low dose irradiation treatment increases the antibiotic resistance of bacteria (Teufel, 1980). Antibiotic resistant strains of pathogenic bacteria were not found more resistant to irradiation. Nalidixic acid resistant *Salmonella* isolates have been shown to have an increased sensitivity to irradiation (Niemira and Lonczynski, 2006) but no correlation was found between the D_{10} values of *Salmonella* isolates and their resistance to ampicillin, chloramphenicol or gentamicin (Niemira et al., 2006). The D_{10} values of strains of *Salmonella* Typhimurium DT104, an antibiotic resistant pathogen isolated from ground meat fell within the previously reported range of radiation D_{10} values in meat products (Rajkowski et al., 2006).

4.4. Effects on toxin production

Some past studies indicated increased production of aflatoxin when spores of *Aspergillus flavus* or *A. parasiticus* were irradiated. However, those studies were performed under highly unpractical conditions. It is well established now that the size of the inoculum has a decisive influence on mycotoxin formation and this was not taken into account by those early studies evaluated e.g. by Ingram and Farkas (1977). Toxin production is apparently suppressed when the number of inoculated mould spores per unit volume of substrate exceeds a certain level (Karunaratne and Bullerman, 1990). A medium inoculated with a high number of mould spores will develop less aflatoxin than one inoculated with lower number of spores. A reduction in the number of unirradiated spores by several $\log_{10}$ cycles by simple dilution caused considerable increase in toxin production (Odamtten et al., 1987; Sharma et al., 1980). Experiments carried out under conditions simulating practical application have either found unchanged or, more frequently, decreased production of mycotoxins in irradiated foods (Chang and Markakis, 1982; Chiou et al., 1990; Ito et al., 1994). Results of many repeated cycles of sublethal irradiation and growth of various strains of *A. flavus* showed that such recycling treatment more frequently led to a decrease or complete loss of aflatoxin production more frequently than to an increase (Frank et al., 1971). According to Diehl (1995) "*there are no indications that any food irradiated and stored under conditions prevailing in practice would be at risk of increased formation of mycotoxins*". Sharma (1998) concluded that food irradiation experiments have not provided any evidence of increased risk of mycotoxin formation in irradiated food and that therefore these do not pose any specific hazard in relation to mycotoxin production.

More recently Kottapalli et al. (2006; 2003) tested treatments up to 10 kGy to reduce the colonisation of cereals with mycotoxin producing fungi, 6 to 8 kGy for instance permitting a significant reduction of the risk of mycotoxin accumulation during barley germination for malt production.

4.5. Effects on the development of radiation resistance

The effect of treating several species important in food hygiene with repeated doses of 2 kGy of γ-radiation alternating with periods under growth conditions was published by Erdman and co-authors as early as 1961(Erdman et al., 1961). An increase of radiation resistance was found with some of the bacterial species when exposed to up to 12 cycles of irradiation and culturing the surviving cells. Similar observations were reported by Idziak and Incze (1968) and Licciardello et al. (1969). Other recycling treatments failed to increase radiation resistance of salmonellae or moulds (Ley et al., 1970; Münzner and Diehl, 1969) and actually resulted in reduced resistance.

The 2003 SCF Opinion states, in relation to the question of multiple cycles of irradiation: "*Many cycles of heat treatment can achieve the same objective but no problems have appeared so far in pasteurization plants*" (Corry and Roberts, 1970; SCF, 2003).

More recently Levanduski and Jaczynski (2008) found that *E.coli* O157:H7 experimentally inoculated in ground beef increased its radio-resistance when submitted to four following cycles of e-beam irradiation at sub-lethal levels (D_{10} values increased from 0.24 to 0.63 kGy). However, a fifth irradiation cycle did not result in a further increase of the resistance. Harris et al. (2009) submitted cultures derived from a single colony isolate of E. coli to 20 iterative cycles of gamma-irradiation and outgrowth. Irradiation dose was increased from 2 kGy (first cycle) to 10 kGy (last cycle). Results from the study indicated a progressive increase of the radiation resistance of the microorganisms by a factor of 1,000-5,000 when subjected to irradiation at 5 kGy. Further studies in one colony indicated that the acquired resistance was maintained when growing the bacteria for 100 generations.

In conclusion, under industrial conditions of food irradiation, no situation is conceivable whereby a population of microorganisms would be repeatedly resuscitated after sublethal irradiation by providing an optimal growth medium and optimal temperatures. The appearance of genotypes with higher radiation resistance in some of these recycling experiments is therefore of more academic than

practical significance. Some food irradiation facilities and many other radiation-emitting source have been in operation for many years now and no evidence for increased occurrence of resistant strains in the environment of these facilities has been found.

4.6. Effects on changes in diagnostic characteristics

This question applies to all processes that may cause sublethal damage on microorganisms. Survivors of sublethal irradiation are usually more demanding in their growth requirements, due to genetic damage. However, the changes are less serious than might be thought because changes are usually temporary and can be reversed by resuscitation (Ingram and Farkas, 1977; Teufel, 1980). A recent study demonstrated that spores of *Bacillus anthracis* γ-irradiated in suspension in their virulent and live stage by a high dose (25 kGy) could be detected by real-time PCR or sandwich ELISA, without decreasing the sensitivity of either type of diagnostic assay (Dauphin et al., 2008). In a similar previous study on the inactivation of spores of avirulent strains of *Bacillus anthracis* either by high dose or autoclaving, Dang et al. (2001) reported a decrease in the sensitivity of real-time PCR and antigen detection by ELISA with monoclonal and polyclonal antibodies. After inactivation by 25-28 kGy, PCR detection required five additional cycles for detection of a positive sample and nine additional cycles were required in the case of autoclaved samples compared with untreated spores samples.

When using PCR for microbial detection in irradiated samples, proper methods of culture enrichment and DNA extraction are needed, since most of PCR techniques are unable to distinguish between DNA originating from viable cells and DNA released from dead cells. Trampuz et al. (2006) have studied the effect of gamma irradiation on amplifiable DNA (using quantitative PCR amplification) of *Staphylococcus epidermidis* and *Escherichia coli* and on the cell viability. They showed that gamma irradiation (doses up to 12 kGy) has little effect on amplifiable 16S rRNA genes present within inactivated bacterial cells, resulting in amplification of DNA from dead cells. DNA lesions are capable of stopping a thermostable polymerase on the DNA template (Lim et al., 2006; Sikorsky et al., 2004), and therefore too many lesions will result in reduced PCR amplification of the target sequence, and underestimation of pathogen load (if using quantitative methods). Lim et al. (2008) found that *Salmonella* counts were underestimated in irradiated samples (increasing the minimum level of detection) because the available template DNA molecules were reduced, subsequently increasing PCR cycle threshold. It should be noted that no resuscitation step was employed. Other key aspects in microbial detection are the target fragment chosen for amplification, the number of copies of the gene present in the cytoplasm and its sensitivity to radiation, which depends on the base composition and/or the spatial localization of the gene on the chromosome (Lim et al., 2007; Lim et al., 2006).

4.7. Effects on organoleptic characteristics of food

Fears that irradiation makes spoiled food marketable by reducing the perceptibility of spoiled state are unfounded. Irradiation cannot improve the odour, taste or visual appearance of spoiled food. If non-spoiled foods are treated with a non-sterilizing dose, these foods may exhibit somewhat changed spoilage characteristics even if finally spoils. Other non-sterilising food treatments have similar effects when eliminating pathogens of significance in food (e.g. heat-pasteurization). The SCF (2003) stated that "*The concern over the misuse of irradiation to sanitize unacceptably contaminated spoiled food has no real basis, as irradiation does not restore the appearance and the organoleptic characteristics of the spoiled food*".

Inappropriate uses of processing to disguise microbial contamination could still be detected by DNA hybridization techniques in samples even in the absence of viable bacteria (Diehl, 1995; Rowe and Towner, 1994).

The considerations in Sections 4.1 to 4.7 reinsure and substantiate the statements of the SCF Opinion of 1986 and 2003 (SCF, 1986, 2003) made regarding microbiological safety of proper use of food irradiation.

5. Update of the scientific knowledge on irradiation of the foodstuffs listed in the previous SCF Reports/Opinions

The scientific literature available since the publication of the past SCF Reports/Opinions has been reviewed in order to identify new scientific data on irradiation of the specific food classes/commodities mentioned in the terms of reference of the mandate. Appendix C to this Opinion contains detailed information on the objectives and limitations of irradiation in the different food classes/commodities, on the hazards identified in those food classes/commodities, with special attention to new or emerging hazards compared to the past, on the efficacy of irradiation for controlling such hazards and, where appropriate, on the comparison and association with other methods. In the sections below a summary of the most relevant information extracted from Appendix C to the Opinion is provided for each of the food classes/commodities evaluated. The summaries include, in particular, information on the new and emerging hazards, on their trend and on the new data available from scientific literature whenever this is not in line with knowledge already available in the past, especially in terms of efficacy and microbiological safety of food irradiation. It should be noted that the relevant scientific publications are not all quoted in the text for the sake of conciseness, but can be found in the detailed sections for each food category in Appendix C.

In light of the information reported, the efficacy of the doses recommended by the SCF in the past in reducing pathogens potentially contaminating the food classes/commodities under evaluation is discussed.

5.1. Fruits

In 1986, the Scientific Committee for Foods (SCF, 1986) recommended that fruits could be irradiated at doses up to 2 kGy. No rationale for this recommended dose was given in this SCF report.

Since 1986, ready-to-eat fruit products such as fresh fruit salads and non pasteurized fruit juices have caused several food-borne outbreaks (CDC, 2009b; Lynch et al., 2009; Vojdani et al., 2008), several of them linked to imported fruits. The agents implicated were mostly *Salmonella* and *E. coli* O157, but also parasites and viruses. Nevertheless, in the EU, fruit berries and juices and products thereof still represent a very minor part of the total reported food-borne outbreaks (2 outbreaks out of 2,025 verified food-borne outbreaks in the EU in 2007 (EFSA, 2009)). Outbreaks linked to fresh fruits or juices have been of concern mostly in the US where fruits and nuts were involved in 2006 16% of cases from outbreaks linked to a single food vehicle (CDC, 2009b). Outbreaks linked to fruits have presumably increased since 1986, potentially because of the development of new, ready-to-eat products. It should be noted that more and more fruits are offered to consumers as ready-to-eat products (e.g. unpasteurised fruit juices, fruit salads), having received no treatment that would significantly reduce numbers of food-borne pathogens. More outbreaks might also have been detected as a consequence of the improvement of the surveillance systems (e.g. PulseNet).

Since 1986 new work concerning irradiation of ready-to-eat fruits has been performed. D_{10} values obtained for the pathogenic bacteria *Salmonella*, *E. coli* O157 and *Listeria monocytogenes* were similar to those reported in Chapter 3 of the present Opinion (Arvanitoyannis et al., 2009). Hepatitis A virus was more resistant than bacteria with a D_{10} value on strawberries close to 3 kGy. As for other foods, irradiation can be combined with mild heat treatments.

The irradiation doses applicable on fresh fruits and processed fruit products are limited by their impact on quality, depending on the type of product (Arvanitoyannis et al., 2009). Irradiation alone

should permit 2 to more than 5 $\log_{10}$ reduction of pathogenic, non spore forming bacteria, in some fresh and many processed fruits. However, for the most radiation sensitive fruits, less than 1.5 $\log_{10}$ reduction should be expected. Concerning pathogens more resistant to irradiation, such as viruses, the doses applicable to most fresh fruits and fruit products will be of limited efficacy.

In many cases food irradiation should be more efficient than surface decontamination of fruits with chemical agents, which reduces pathogens by at most 2 $\log_{10}$ (WHO, 1998). Unlike surface decontamination, irradiation can be applied on the product in its final package, precluding the risk of re-contamination. Irradiation is presumably unique in this respect in the case of fresh fruits. In the case of mildly processed fruits such as juices, high pressure treatments could offer similar advantages.

The 2 kGy limit recommended in SCF (1986) should permit decimal reductions in the number of non spore forming pathogenic bacteria ranging from around 2 (e.g. *L. monocytogenes* considering the highest D_{10} valus reported) to 5 or more (e.g. *E.coli* O157) according to Arvanitoyannis et al. (2009), depending on the pathogen. However, due to the very broad diversity of fruits species and fruits products, and their very diverse sensitivity to irradiation, such dose may not always be tolerated. In contrast, some products might tolerate higher doses.

5.2. Vegetables

In 1986, the Scientific Committee for Foods (SCF, 1986) recommended that vegetables could be irradiated at doses up to 1 kGy. No rationale for this recommended dose was given in this SCF report. In particular, it is not clear why the recommended dose for vegetables was different from that for fruits.

Reports on food-borne outbreaks linked to vegetables have increased since the 1986 SCF Opinion, as observed for fruits (CDC, 2009b; Lynch et al., 2009). Similarly to fruits, these outbreaks have frequently been caused by pre-washed and packaged vegetables, as well as ready-to-eat, fresh-processed products, and sprouted seeds. In addition, global trade of vegetables was the cause of several large multinational outbreaks (Lynch et al., 2009). Most outbreaks were caused by *Salmonella*, *Shigella*, *E. coli* O157 and other EHEC sevovars. However, in the EU, in spite of recurrent and large outbreaks, vegetables and juices and other products thereof still represent a minor part of verified food-borne outbreaks (0.79% in 2007 (EFSA, 2009)), and the food-borne pathogens which have caused these outbreaks are very rarely detected in fresh produce. In contrast, the situation is markedly different in the US where leafy vegetables represented, in 2006, 17% of cases from outbreaks linked to a single food vehicle (CDC, 2009b).

As observed for fruits, D_{10} values of pathogens were within the range of those reported in Chapter 3 of the present Opinion (Arvanitoyannis et al., 2009).

The efficacy of irradiation and its limits are the same for vegetables as for fruits. As for fruits, vegetables are increasingly used by consumers as ready-to-eat products. However, unlike acid fruits, most vegetables support growth of bacterial pathogens whenever relative humidity and temperature are high enough. Sprouted seeds represent a specific case as irradiation treatments were tested both on the dry seeds before sprouting and on the final sprouts. Efficacy of irradiation to inactivate pathogens present on sprouted seeds is the same as for other fresh vegetables whereas on dry seeds it is similar to that on cereal (see Section 5.3 below).

A dose of 1 kGy would allow only a limited reduction in the number of relatively resistant food-borne pathogens (e.g. *L. monocytogenes* considering the highest D_{10} values reported) but may permit higher reductions (up to 4 - 5 $\log_{10}$ according to some studies quoted by Arvanitoyannis et al. (2009)) of more sensitive pathogens (e.g. pathogenic *E. coli*). New evidence suggests that some vegetables could tolerate higher doses without quality loss.

5.3. Cereals

Irradiation of cereals at doses up to 1 kGy was proposed by the SCF in 1986 (SCF, 1986). No objective for the application of this dose on cereal was given, but irradiation was presumably intended for disinfestation purposes. Cereals, grains and dry legume seeds are usually consumed after a wide range of processing operations. Irradiation, tested at doses between 1 and 10 kGy, can modify the quality and technological properties of cereals and cereal products positively or negatively.

The major safety concern since 1986 for cereals has been mycotoxin producing fungi. Treatments up to 10 kGy have been tested to reduce the colonisation of cereals with mycotoxin production fungi, 6 to 8 kGy for instance yielding a significant reduction of the risk of mycotoxin accumulation during barley germination for malt production (Kottapalli et al., 2006; Kottapalli et al., 2003). However, such doses have no impact on toxins already present in the cereals.

Processed cereals have caused food-borne outbreaks due to pathogenic spore forming bacteria such as *Bacillus cereus* in cooked rice (EFSA, 2005b). On raw rice, 3.2 and 7.5 kGy permitted a reduction of respectively 1.5 and 2.6-3.0 $\log_{10}$ reduction in *B. cereus* spores (Sarrias et al., 2003). Irradiation of cereals to reduce contamination with bacterial spores is presumably possible. However, risk of *B. cereus* poisoning occurs in large part during handling and storage of the processed product (EFSA, 2005b). Other decontamination methods would presumably not permit a similar reduction of bacterial spores without modification of cereal quality.

Processed cereal, nuts or grains have been a cause of salmonellosis (CDC, 2009a; de Jong et al., 2001). On dry seeds other than cereals, irradiated to reduce pathogenic bacteria before production of sprouts, D_{10} values for *Salmonella* strains were between 0.74 and 1.1 kGy (Rajkowski et al., 2003), higher than those reported in most foods, presumably because of the low humidity present on the surface of seeds (see Chapter 3 of the present Opinion). Decontamination of seeds intended for sprout production with chemical agents is of limited efficacy (Weissinger and Beuchat, 2000). D_{10} values ranging from 0.6 to 0.8 for *Salmonella* in peanut butter with an a_w of 0.51 were reported (Hvizdzak et al., 2010).

Irradiation of cereals at a dose of 1 kGy would allow only a limited reduction in the number of food-borne pathogens and would not reduce mycotoxigenic fungi. Evidence suggests that some cereals tolerate higher doses without quality loss.

5.4. Starchy tubers

Food irradiation of starchy tubers up to a dose of 0.2 kGy was proposed by SCF (1986). In that report, SCF proposed such irradiation doses for sprouting inhibition or disinfestation purposes. Since 1986, published studies on irradiation of starchy tubers concerned mostly sprouting inhibition, shelf-life extension and disinfestation, but not the reduction of food-borne pathogens.

Starchy tubers are normally consumed after heat treatments and have rarely been the cause of food-borne disease. In one instance handling raw potatoes contaminated by *E. coli* O157 was presumably the cause of some human infections in England (Morgan et al., 1988). Cooked potatoes kept unrefrigerated before consumption have also been the cause of botulism (Nguyen-the and Carlin, 2000). In such examples, food-borne diseases could have been prevented by adequate hygiene or correct food handling and temperature control.

Starchy tubers are also an important source of starch used in the food industry. Starch can be a significant source of spores of pathogenic bacteria such as *Bacillus cereus* in processed foods (Guinebretiere et al., 2003; Guinebretiere and Nguyen-The, 2003). Irradiation could presumably reduce the number of bacterial spores in starch at doses similar to those used for other dry foods.

5.5. Spices and condiments

Irradiation of spices and condiments at doses up to 10 kGy was proposed by the SCF (1986). No objective for the application of this dose on spices and condiments was given in the SCF report, but irradiation was presumably intended for the reduction of microbial load. The landmark directives of the European Union in February 1999 allowed irradiation of spices and dry aromatic herbs up to 10 kGy dose levels, beginning in 2000. Prior to and after this date, clearances of many countries worldwide have been granted for spices and condiments.

Spices and condiments, in their natural state, contain a large number of microorganisms. Irradiation is a recognized and feasible method for reducing the microbial load of spices and condiments with minimal effects on their sensory properties. Among the spore-forming bacteria, *Bacillus cereus* and *Clostridium perfringens*, which are capable of causing gastroenteritis when ingested in large numbers, are frequently found in spices, but usually in low numbers. In extreme cases, however, *Bacillus cereus* counts up to 10^5 CFU/g have been found. Several other *Bacillus* spp., which are opportunistic pathogens, are more frequently isolated from spices.

Salmonellae have been found, albeit infrequently, in a variety of spices. Their presence is of special concern when spices are used in foods that are consumed raw, or when the spices are added to foods after cooking. Indeed, peppers have been implicated in salmonellosis outbreaks in Canada, Norway and Sweden, respectively. In 1993, a nationwide outbreak of salmonellosis occurred in Germany following ingestion of paprika and paprika-powdered potato chips contaminated with a great variety of *Salmonella* serovars. In 2009, due to a multi-state *Salmonella* Rissen outbreak in the United States numerous spice products were recalled, and another multi-state outbreak of *Salmonella* Montevideo infection by salami products was confirmed to be related to their ingredients, black and red peppers, as a source of contamination[13].

Mould counts of spices and herbs may reach the 10^5 propagules per gram level, and a relatively high incidence of toxigenic moulds has also been found. Since 1986 mycotoxin producing fungi have been an increasing concern.

Recent publications on irradiation of spices and condiments did not produce entirely new aspects or type of information as compared with the extensive former literature, and they proved the technological feasibility of radiation processing of additional items.

Irradiation of spices and condiments with a dose in the range of 5-10 kGy allows effective reduction of food-borne pathogens. The exact dose applied would depend on the contamination and the desired inactivation factor (Farkas, 1998).

Considering its antimicrobial activity and relatively minor effects on quality, irradiation is the most effective method of microbiological decontamination for this class of food ingredients (Farkas, 2000).

5.6. Fish and shellfish

In 1986, the Scientific Committee for Foods (SCF, 1986) recommended that fish and shellfish could be irradiated at doses up to 3 kGy.

Irradiation of fish and shellfish is intended to extend shelf-life, reduce pathogen load and inactivate parasites. Another application is the disinfestation of dried fish and smoked fish to prevent deterioration due to insect damage (Boisot and Gauzit, 1966; Venugopal, 2005). Irradiation has been applied to fresh, frozen as well as dried fish, fish products, and shellfish.

[13] See http://cdc.gov/salmonella/montevideo/index.html, accessed on 21 September 2010.

Biological agents responsible for food-borne outbreaks are commonly found in fish and shellfish. According to the EFSA (2009), fish and fish products were responsible for 6.4% of verified outbreaks in the EU in 2007, including those caused by biotoxins and amines. A number of reports dealing with *Vibrio, Salmonella* and viruses as health risks in fish and shellfish (molluscs) have been published (Dalsgaard, 1998; Igbinosa and Okoh, 2008; Lees, 2000; Lyytikinen et al., 2006; Norhana et al., 2010; Robertson, 2007; Su and Chengchu, 2007). Disease caused by pathogenic Vibrios, *Listeria monocytogenes,* viruses and parasites is commonly related to consumption of raw products.

Literature review from 1986 reveals that D_{10} values needed to reduce or eliminate bacterial pathogens (such as pathogenic *Vibrio, Salmonella,* pathogenic *Escherichia coli* etc.) are similar to those reported previously. As for other foods, pathogenic bacteria were more resistant to irradiation in frozen than in fresh fish and fish products. Most studies indicate that irradiation at doses recommended by the SCF (3 kGy), should yield 2 to 5 $\log_{10}$ reduction of pathogenic, non spore forming bacteria for the majority of fish and fish products. Other technologies, such as high hydrostatic pressure, can yield similar reductions and offer similar technological advantages with adequate sensory properties. Viruses, biotoxins and some parasites (*Anisakis simplex*), show low susceptibility to irradiation. Third stage larvae of *Anisakis simplex* have a high degree of radioresistance and it is not possible to inactivate the parasite at the doses normally used and possibly even at doses higher than 6 kGy (Loaharanu and Murrell, 1994; Padovani et al., 2005; Seo et al., 2006). In contrast freezing can inactivate *Anisakis* in raw fish.

The irradiation dose applicable to fish and fish products is limited by its impact on quality. Fat content is a key factor because of sensory problems arising when fish has high fat percentage. In order to determine the best irradiation dose to prevent sensory changes, a specific study should be conducted for each fish/shellfish species. Sensory changes depend on the type of fish and can be reduced by vacuum-packaging. However vacumm-packaging must be associated to refrigeration or freezing to prevent the risk of *Clostridium botulinum*.

A dose of 3 kGy permits a significant reduction of vegetative bacterial pathogens in fish (around 2 to 5 $\log_{10}$ reduction), but considering the diversity and differences in composition in fish and fish products and pathogen sensitivity to irradiation, the dose applicable should be adapted to the product and the pathogen reduction required.

5.7. Poultry

Irradiation of poultry up to an overall average dose of 7 kGy was proposed by the SCF (1986) with the purpose to improve microbiological safety.

Campylobacter spp. continue to be a major cause of food borne disease in the EU. Contaminated poultry meat, in particular, has been identified as a major source of human campylobacteriosis. In the EU, in 2008, approximately 30% of raw poultry meat was found to contain this pathogen, compared to 5.1% being positive for *Salmonella* spp. (EFSA, 2010).

Decontamination of poultry carcasses by chemical treatments has been proposed. The EFSA Panel on Biological Hazards (BIOHAZ) evaluated the efficacy of peroxyacids but from the data available could not confirm the efficacy of these substances (EFSA, 2005a).

Further research has confirmed previous findings on the radiation-response of the main vegetative pathogens (thermophilic *Campylobacter* spp., *Salmonella*, *Listeria monocytogenes*, *E. coli* O157:H7) when treated in poultry meat (Dion et al., 1994; Gursel and Gurakan, 1997; Patterson, 1995; Sanos et al., 2003; Thayer et al., 1995; Thayer et al., 1998). *Listeria monocytogenes* was generally found to be more radiation resistant than the other vegetative pathogens, with D_{10} values of around 0.70 kGy being reported in cooked turkey breast nuggets (Thayer et al., 1998).

Based on the scientific evidence, the current recommendation for an overall average dose of 7 kGy would be sufficient to give at least a 5-$\log_{10}$ reduction in the number of vegetative pathogens in frozen poultry products. Lower doses would be sufficient to achieve the same reduction in chilled poultry products.

5.8. Fresh red meat

Irradiation of fresh meat up to an overall average dose of 2 kGy was proposed by the SCF in 1986 (SCF, 1986).

Since 1986, the implication of meat in food-borne illness has declined, but remains a concern, particularly with respect to salmonellae, verocytotoxigenic and enterohaemorrhagic *E. coli* and *L. monocytogenes*.

Publications appeared in recent years with regard to irradiation of fresh meat confirmed the validity of the previously established principles and sensitivity of microorganisms (*Salmonella* spp., *E. coli* 0157:H7, *Yersinia* spp., *Listeria monocytogenes*) to treatment with irradiation, expressed as D_{10} values. D_{10} values identified by studies on fresh red meat for the different food-borne pathogens are in line with those described above for the same agents on poultry meat and more generally in Chapter 3 of the Opinion (Arthur et al., 2005; Badr, 2004; Bari et al., 2006; Black and Jaczynski, 2008; Gezgin and Gunes, 2007; Kiss et al., 2001; Rajkowski et al., 2006; Schilling et al., 2009; Sommers and Niemira, 2007; Sommers and Novak, 2002).

The radiation dose required to reduce numbers of a pathogen is calculated from the D_{10} value and whether the meat is chilled, frozen or dried. There seems to be no reason to limit the radiation dose for fresh meat to 2 kGy.

Irradiation of fresh meat can change colour, odour and taste. These changes can be minimised by modified atmosphere packaging and reduced fat levels, and largely prevented by irradiating in the frozen state.

Some studies investigated the use of e-beams to decontaminate carcass surfaces and in the US a petition is pending for approval for this use. Also, it has been requested to consider its use to inactivate *L. monocytogenes* on packaged products allowing its growth.

5.9. Camembert cheeses manufactured from raw milk and soft cheeses in general

SCF (1992) agreed that the treatment of Camembert cheeses manufactured from raw milk with gamma irradiation at doses up to 2.5 kGy was acceptable from a health point of view. The main objectives considered by the SCF were reduction of food-borne pathogens and extension of shelf-life.

The use of raw milk in the production of soft cheese, faulty pasteurization or equipment, post-processing contamination or cross contamination are still contributing factors for reported outbreaks in which soft cheeses are involved.

The pathogenic microorganisms or toxin-producing microorganisms considered in studies on irradiation of cheeses are: *Listeria monocytogenes*, pathogenic *Escherichia coli*, *Salmonella*, *Clostridium*, *Staphylococcus*, fungi (mycotoxins), *Brucella* and *Mycobacterium*. More recently, *B. cereus* has also been identified as a pathogenic microorganism that can be transmitted by some types of cheeses. These studies, done on soft cheeses other than camembert, confirmed previous findings on the radiation-response of the main pathogens.

Cheeses are also incorporated in ready-to-eat foods, which may represent a new issue since the previous SCF Opinion. In various ready-to-eat foods containing cheeses, different authors have reported D_{10} values for *Listeria monocytogenes* ranging from 0.27 to 1.38 kGy in function of the substrate or food commodity (Bougle and Stahl, 1994; Sommers and Boyd, 2005; Tsiotsias et al., 2002). Methods other than irradiation were also tested to reduce food-borne pathogens in cheeses. High hydrostatic-pressure treatment at 400 MPa was found to cause a reduction of 7 logs of *E. coli* populations in fresh cheese (Trujillo et al., 2000).

According to the published literature, 2.5 kGy would reduce the number of food-borne vegetative pathogens by about 3 $\log_{10}$ (or less for the relatively more resistant pathogen *L. monocytogenes*). There is evidence that some cheeses tolerate higher doses without quality loss.

5.10. Frog's legs

SCF (1998) recommended that frog's legs could be irradiated at doses up to 5 kGy. Frogs legs are commonly contaminated with *Salmonella* and other pathogens, such as *Escherichia coli* and *Staphylococcus aureus*. Although frog's legs are cooked before consumption, there is a risk for cross-contamination.

From information collected in the scarce recent scientific literature on irradiation of frog's legs, several log units reduction of pathogenic microflora usually present in deep frozen product is easily achieved by irradiation at an average dose of 5.0 kGy.

5.11. Shrimps

The SCF (1998) recommended that shrimps could be irradiated at doses of 5 kGy.

Shrimp is frequently imported from tropical and subtropical areas and reports indicate that the product does not always meet the microbiological standards set for EU producing countries (Beckers et al., 1981; Cann et al., 1981; Hatha et al., 2003; Pinu et al., 2007), because either of contaminated production sites or unhygienic processing conditions.

Major microbial hazards to be considered within this category of food include: *Vibrio parahaemoliticus*, *V. vulnificus*, other pathogenic *Vibrio, Staphyloccoccus aureus, Listeria monocytogenes*, *Aeromonas hydrophila*, *Shigella* spp. and *Salmonella*.

Nerkar and Bandekar (1990), Rashid et al. (1992) and Ito et al. (1993) studied the effect of γ-irradiation on frozen shrimp for pathogen decontamination. The dose needed to reduce *Vibrio* and *Aeromonas hydrophila* by 10^{-4} per gram was about 3 kGy in frozen shrimp, while 3.5 kGy was required for *L. monocytogenes* and 1.0 kGy for *Salmonella*. γ-Irradiation of frozen shrimp with either 2.5 and 4.7 kGy reduced mesophilic bacteria contamination from 10^3 CFU/g to low and nondetectable levels, respectively (Sinanoglou et al., 2007).

Shrimp is considered separately from fish and shellfish given that certain pathogens (i.e *Listeria monocytogenes*) require doses in excess of 3 kGy for several $\log_{10}$ reduction. Irradiation with doses of 5 kGy is considered to be an effective decontamination method.

5.12. Gum Arabic

Irradiation of gum arabic was evaluated by the SCF in 1998, which recommended the dose of 3 kGy for irradiation of this product (SCF, 1998). The reason behind the request was microbial decontamination of gum arabic when it was to be used in pharmaceutical preparations, especially due to the unhygienic conditions of collection, preparation, storage and transport in the country of origin.

However, gum arabic is also used in the food industry for several purposes, such as a component of emulsifiers, flavoring agent, humectant, thickener, surface-finishing agent, sugar-crystallisation retardant, confectionery beverage, and encapsulation of bakery products (Zaied et al., 2007) and it is included in the EU list of allowed food additives provided by Directive 1995/2/EC[14].

Microbiological agents of potential concern for this food category mainly include unspecific agents originating from the external environment and cross-contaminating gum Arabic during its production and shipping.

There is a lack of scientific studies concerning the application of irradiation to gum arabic. Zaied et al. (2007) concluded that the optimal dose to be used for decontamination of gum arabic for use both in the food industry and medicine would be 5.0 kGy, which in their experiment was sufficient to reduce counts of bacteria, including some pathogenic ones (i.e. *B. cereus* and *C. perfringens*), by 1 to 3 $\log_{10}$ units.

In spite of the lack of published information, 3 kGy will presumably only permit a limited reduction of pathogens in a dry product such as gum arabic.

5.13. Casein and caseinates

SCF (1998) recommended that casein and caseinates could be irradiated at doses up to 6 kGy as a mean to decontaminate these casein products, to avoid infections of the consumer.

Casein products can be used in foods for sensitive consumers such as infant food formulas. Contamination of infant food formulas with *Cronobacter sakazakii* has been associated with numerous cases of meningitis and necrotizing entercolitis in infants (EFSA, 2007a).

A number of studies published since the previous SCF Opinion have determined the radiation resistance of *C. sakazakii* in powdered infant formulas. In most cases, reported D_{10} values range from 0.76 to 1.98 kGy, depending on the strain and on the length of time the organism was stored in the powder post-treatment (Lee et al., 2006; Osaili et al., 2008a; Osaili et al., 2007). Dry storage appears to increase the radiation resistance of the pathogen in the powdered formulas (Osaili et al., 2008b). One study reported a higher D_{10} value of 4.83 kGy (Hong et al., 2008). The authors suggest the much higher D_{10} value in their study could be due to differences in the formulation and this would need to be confirmed through further investigation.

However, other approaches than decontamination of the dried formulas could be explored. For instances, studies carried out by Pina Perez et al. (2007) indicated that pulsed electrical fields were effective against *C. sakazakii* in the re-hydrated infant formulas. This could represent an alternative to the use of irradiation.

According to the available studies, there is not reason to modify the former consideration about irradiation of caseinates. Inferring from the results obtained in dried foods, 6 kGy should reduce the number of vegetative food-borne pathogens by several $\log_{10}$ units, unless the unusual resistance of *C. sakazakii* reported in the above study was confirmed.

5.14. Egg white and eggs in general

In the 1990s, the use of irradiation for egg white, whether liquid, frozen or dehydrated, was requested in order to assure a bacteriological quality (both for pathogen reduction and shelf-life extension) as

[14] European Parliament and Council Directive No 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners. OJ L 61, 18.3.1995, p.1-40.

needed by the food industry and to avoid secondary contamination of the decontaminated product during packaging, storage and transport (SCF, 1998). Besides these objectives, irradiation has also been used for the reduction of allergenicity of eggs and products containing eggs (Seo et al., 2004) and proposed for improving egg white foaming ability and quality of final bakery products (Song et al., 2009). The SCF (1998) only considered egg white, but other egg products exist (such as egg yolk, egg blends, in-shell eggs) which might be submitted to irradiation.

The microorganisms of concern in this category of food are represented by bacteria of genera *Salmonella*, *Campylobacter* and *Staphylococcus* (Alvarez et al., 2006; Badr, 2006). More recent studies are focused on *Salmonella* control, mainly in shell eggs, being probably the most important target for this food category. In the case of liquid egg, the pasteurization process should destroy the microorganisms, although it does not inactivate *Bacillus cereus* spores, which are of concern for some companies using liquid eggs for the production of cream-caramel or other processed foods (Collado, 2008).

Recent studies reported D_{10} values for *Salmonella* in shell eggs and liquid eggs between 0.5 and 0.65 kGy (Al-Bachir, 2005; Alvarez et al., 2006), with some serovars (e.g. Senftenberg) being more resistant. The indicated D_{10} values for *Salmonella* in shell and whole eggs are in the range of those reported in Chapter 3 of the present Opinion.

The combination of irradiation with other technologies of preservation could be applied to some egg products in order to achieve a higher reduction of vegetative pathogens. The effect of combining irradiation, heat (i.e. irradiation followed by heat) and a number of additives on *Salmonella* Enteritidis and *Salmonella* Senftenberg inoculated into liquid whole egg was investigated by Alvarez et al. (2007a; 2007b). Results indicated synergistic effects of those treatments in the reduction of the studied microorganisms.

The dose of 3 kGy would reduce the number of food-borne vegetative pathogens. However, irradiation at this dose would not always be sufficient to achieve the 5-$\log_{10}$ reduction obtained through pasteurisation. Due to the very diverse sensitivity of egg products to irradiation, application of doses higher than 3 kGy may result in a loss of quality in certain products but may be tolerated by others.

5.15. Cereal flakes

The SCF (1998) concluded that irradiation of cereal flakes at 10 kGy was acceptable as an appropriate measure to protect the health of the consumer. In particular, irradiation was proposed as a method capable of destroying the spores of pathogenic *Bacillus* species, which could contaminate cereal flakes and germ intended for use as ingredients in yoghurts made from whole milk. An earlier publication from France (Hendon, 1984) stated that muesli-like cereal products mainly consisting of cereal flakes were good candidates for radiation decontamination. Since then no publications on the effect of irradiation on food-borne pathogens in cereal flakes was identified.

5.16. Rice flours

The SCF (1998) concluded that irradiation of rice flour with up to 4 kGy is acceptable. In particular, irradiation was proposed "*for rice flour used in special foods for infants and other sensitive sections of the population, requiring food of special microbiological quality not achievable with normally available products"* in order "*to achieve the desired microbiological purity of rice flour when used as an ingredient of baby foods during their manufacture"*.

No new publications on the effect of irradiation on food-borne pathogens in rice flour were identified. Some works on irradiation to inactivate pathogens in infant food formulas, which could contain rice flour as an ingredient, are presented in Section 5.13 on casein and caseinates.

5.17. Blood products

According to SCF (1998), "*a considerable tonnage of blood collected from animals killed in slaughterhouses was wasted because of its poor initial microbiological quality due to the method of collection and the poor hygiene conditions existing in the small slaughterhouses. As blood, plasma and packed cell mass, when dehydrated, constituted a source of nutritious proteins used by the food industry for the production of human food, some method of decontamination was needed to reduce wastage of this protein and to reduce costly imports of other protein sources*". The SCF (1998) concluded positively on the irradiation of blood products at the dose of 10 kGy, indicating however that doses adequate to kill bacteria would not inactivate any contaminating viruses or prions.

In principle, all the microbiological hazards identified for meat can be also considered for this food category. While the list of hazards is long, there have been very few incidents in foods traced to use of blood products in recent years, after marked improvements in hygienic collection of blood.

An earlier publication from Hayashi et al. (1991) investigated the use of irradiation to reduce the microbial load in dehydrated porcine blood plasma contaminated by a microbial flora mainly constituted by *B. subtilis*. D_{10} values were 0.82 kGy for γ-rays and 1.06 kGy for electon beams and the authors estimated that the microbial load could be decreased to a count lower than 10/g by irradiating at 10 kGy even if starting from a microbial load of 10^9/g.

Studies due to the concern over carriage in bovine blood of TSE agents confirmed the very high tolerance to radiation of certain TSE agents (scrapie agents in hamsters/mice). Treatment with doses as high as 50 kGy reduced but did not entirely eliminate infectivity (Gominet et al., 2007; Miekka et al., 2003).

No more recent publications on the effect of irradiation on food-borne pathogens in bovine and porcine blood intended for food production were identified after the publication of the SCF report.

6. Other food classes/commodities

Ready-to-eat foods are now very diverse and their consumption has presumably increased since the publication of previous SCF Reports/Opinions. For instance, fresh produce are frequently processed as ready-to-eat products, a large range of ready-to-eat dishes containing cooked meat or cooked sea foods are now proposed and many are a mixture of several types of ingredients. Ready-to-eat foods may represent a specific hazard to consumers whenever they do not undergo a process permitting pathogen reduction, or whenever recontamination with pathogens after processing is possible. Some "traditional" foods are also ready-to-eat (e.g. cheeses, fermented meat product etc.) and may pose the same risk for consumers as more recently developed products. A consequence of this diversity is that the hazards of concern for ready-to-eat foods and the risk for consumers are also very diverse.

In a previous Opinion, EFSA (2007b) stated that "*Previous analysis of sporadic cases and outbreaks of human listeriosis have shown that the foods associated with transmission are predominantly ready-to-eat, capable of supporting the growth of L. monocytogenes"* and recommended that: "*application of GHP in combination with HACCP should be consistently applied to minimise the initial contamination at manufacturing level, and/or reducing the potential for growth*". For instance, ready-to-eat meats, including poultry, have become more popular in recent years and post-processing contamination with *Listeria monocytogenes* is of potential concern in these products. Between 1998

and 2005, at least 41 million kilograms of ready-to-eat meat products have been recalled in the USA due to *L. monocytogenes* contamination (Core, 2005).

The Community legislation (Regulation (EC) No 2073/2005[15]) lays down food safety criteria for *Listeria* in ready-to-eat foods. Producers may seek additional measures to ensure their ready-to-eat products are in compliance with this regulation and irradiation is one option that they may consider.

L. monocytogenes has presumably been the pathogen the most studied in ready-to-eat foods, but it is not the only pathogen of concern. For instance, in ready-to-eat fruit and vegetables products, *Salmonella* and pathogenic *E. coli* have been the major causes of outbreaks (see also Chapter 5).

Extensive research has been devoted to irradiation to improve microbiological safety of ready-to-eat foods, particularly to eliminate non spore-forming pathogens from ready-to-eat meat products, such as bologna, hot dogs, deli turkey and ham. For many of these products, irradiation is an effective treatment that can be used after packaging the final product.

Attention should be paid that in complex foods the efficacy of irradiation may vary with the physico-chemical composition of the various ingredients (see Section 3.2). For instance, in a tortilla based dish, *L. monocytogenes* was two times more resistant in the meat than in the tortilla itself (Sommers and Boyd, 2005). However, several studies summarized recently by Sommers and Boyd (2006), demonstrated that medium (2 to 4 kGy) doses inactivate common food-borne pathogens including *Salmonella* spp., *Staph. aureus*, *L. monocytogenes*, *E. coli* O157:H7 and *Yersinia enterocolitica* in a variety of ready-to-eat food products (e.g. around 3 $\log_{10}$ reduction for 2 kGy). The authors concluded that, when applied as a terminal intervention as part of a HACCP plan, food irradiation could reduce the risk of food-borne pathogens in ready-to-eat foods.

A Joint FAO/IAEA coordinated research project on irradiation to ensure the safety and quality of prepared meals[16], which includes studies over a five year (2002-2006) period on various prepared complex meals (more than 50 different meals were investigated) has shown, too, that irradiation used on its own or in combination with other technologies could significantly enhance the microbiological safety and extend shelf-life of such products (IAEA, 2009). Twelve institutions from different regions of the world participated in the project.

7. Considerations on the categorisation in food classes

The food classes/commodities as listed in the mandate arose historically from a desire to approve categories of food where there were general concerns over microbiological safety, and subsequently in response to particular problems with a number of commodities. At this time, these food categories and food commodities do not represent a systematic classification of foods with respect to irradiation practices.

With regards to the efficacy of irradiation on pathogens, the categories regroup foodstuffs for which irradiation will have a different efficacy to inactivate pathogens, like, for instance, fresh and dry fruits, fresh poultry and frozen poultry etc. In contrast, they separate foodstuffs with very similar properties with regard to efficacy of irradiation, like, for instance, casein, caseinates and rice flour, fresh red meat and fresh poultry etc.

[15] Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338, 22.12.2005, p.1-26 (as last amended).

[16] Irradiation to ensure the safety and quality of prepared meals. Vienna, International Atomic Energy Agency, 2009. ISBN 978-92-0-111108-1.

With regards to the nature of hazards and level of risk for consumers, foods within the same category may represent different microbiological risks. In the case of vegetables, for instance, sprouted seeds represent a different level of risk for bacterial pathogens because sprouting conditions are very favourable for bacterial growth and packaged cut salads favour microbial growth, compared to e.g. raw vegetables which are not normally eaten raw. In contrast, hazard identification and risk factors for vegetables and fruits are similar and in this respect could be considered as a single food class. More generally, the food classes do not identify ready-to-eat foods representing a particular risk for consumers. However, among the food classes considered in this Opinion, fish may deserve a specific attention because it can carry *Anisakis*, which is particularly resistant to irradiation (see also Chapter 3 and Section 5.6).

In addition, some categories are ambiguous, such as "fruit", which could be based on botanical or commercial definition.

The dose of radiation required to reduce a pathogen to an acceptable level is mostly determined by the natural (inherent) resistance of that pathogen.

Hence, categorisation of foods to assess the efficacy of irradiation to inactivate pathogens is not necessary. For each application, the irradiation dose needed to obtain the desired reduction of the pathogen(s) of concern might be inferred from the available scientific information and confirmed by experimental validation and testing. In particular, the dose needed to achieve a given reduction for a given pathogen will be influenced by some physico-chemical conditions of the food (see Section 3.2), i.e. whether the food is chilled, frozen or dried and, to a lesser extent, its fat or protein contents. Beside these factors, the nature of the food has only a limited impact.

The irradiation doses appropriate for a food are a compromise between several objectives and constraints, inactivation of pathogens being only one of them. A list of food categories with recommended doses of irradiation would be too rigid, would oversimplify the determination of the appropriate doses and might be a cause of misuse of irradiation.

Listing main food-borne pathogens for a specific food class/commodity may create the impression that only those pathogens should be of concern, whereas the occurrence of hazards and the importance of the risks may change in the course of time and may be of concern only for some specific categories of consumers.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

- Previous SCF Reports/Opinions addressed many food irradiation objectives in addition to microbiological safety, while this Opinion is only dealing with the efficacy and microbiological safety of food irradiation.

- It is confirmed that there are no microbiological risks for the consumer linked to the use of food irradiation and its consequences on the food microflora.

- Different technologies are currently available and used for the irradiation of food, having to a large extent the same effect on microorganisms. While gamma-rays are produced from a radioactive source, e-beams and X-rays are produced by specific equipment converting other energy sources, without the involvement of any radioactive substance.

- When integrated into an overall food safety management program that includes Good Agricultural, Manufacturing and Hygienic Practices and HACCP, and depending on the dose applied, food irradiation can contribute to improved consumer safety by reducing food-borne pathogens in all the food categories and food commodities addressed by the present Opinion.

- The irradiation dose needed to inactivate food-borne pathogens depends on the targeted pathogen(s), on the reduction required and on the physical state of the food (e.g. water activity, fresh or frozen status), regardless of the food classes as previously proposed.

- Food marketing practices and consumption patterns have changed in recent years and the previous classification did not identify all foods representing a potential high risk for consumers (e.g. some ready-to-eat foods).

- Since the dose applied may be limited by other constraints, the food irradiation process cannot always be designed on the sole basis of the food-borne pathogen of concern.

RECOMMENDATIONS

- Irradiation should be considered as one of several approaches to reducing pathogens in food, e.g. integrated in a multi-hurdle strategy, thereby helping to ensure protection of consumers' health.

- Food irradiation should only be used in conjunction with an integrated food safety management program.

- With regards to efficacy and microbiological safety, the application of food irradiation should be based on risk assessment and on the desired risk reduction rather than on predefined food classes/commodities and doses.

- With respect to the efficacy and microbiological safety of food irradiation, upper dose limits for pathogen reduction should not be specified. Other constraints, such as undesirable chemical changes, will limit the doses applied.

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APPENDICES

APPENDIX A – EU LEGISLATION

Directive 1999/2/EC of the European Parliament and of the Council on the approximation of the laws of Member States concerning foods and food ingredients treated with ionising radiation (also named Framework Directive).

The Framework Directive 1999/2/EC applies to the manufacture, marketing and importation of foods and food ingredients treated with ionising radiation.

Some preconditions apply to the authorisation of food irradiation:

- "*there is a reasonable technological need,*
- *it presents no health hazard,*
- *it is of benefit to the consumers,*
- *it is not used as a substitute for hygiene and health practices or for good manufacturing or agricultural practice.*"

The Directive also sets out the uses for which food irradiation may be used:

- "*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 or organisms harmful to plant or plant products.*"

Foodstuffs can only be treated by a range of sources of ionising radiation: gamma rays from radionuclides 60Co or 137Cs, X-rays (generated from machine sources operating at an energy ≤ 5 MeV) and electrons (generated from machine sources operating at an energy ≤ 10 MeV).

The Directive also establishes that, until the establishment of the Community positive list of foodstuffs that may be treated with ionising radiation, Member States can maintain their existing authorisations, as long as such treatments have been favourably evaluated by the SCF. In addition, Member States can continue to apply national restrictions on irradiated products and on trade on the foodstuffs not indicated in the Implementing Directive 1999/3/EC.

Further measures are included in the Framework Directive as concerns compulsory labelling of irradiated foodstuffs and food containing irradiated ingredients, approval of irradiation facilities in Member States and Third Countries, imports of irradiated foodstuffs, reports to be provided by the Member States and by the Commission on the checks performed on the irradiation facilities and on the products at marketing stage.

Directive 1999/3/EC of the European Parliament and of the Council on the establishment of a Community list of foods and food ingredients treated with ionising radiation (also named Implementing Directive).

The Implementing Directive 1999/3/EC provides an initial Community positive lists of foodstuffs authorised for irradiation treatment and the corresponding maximum doses allowed. It establishes that Member States may not prohibit, restrict or hinder the marketing of such foodstuffs, if irradiated in accordance with the provisions of the Framework Directive 1999/2/EC.

So far, only one food category has been included in the initial Community positive list: "dried aromatic herbs, spices and vegetable seasonings", at the maximum overall average absorbed radiation dose of 10 kGy.

APPENDIX B – INTERNATIONAL BACKGROUND

United Nations organisation activities related to the use of food irradiation

The Joint FAO/IAEA/WHO International Consultative Group on Food Irradiation (ICGFI) was established in 1984 on the initiative of the Directors Generals of FAO, WHO and IAEA, who invited their Member States to consider forming the Consultative Group to further international cooperation in food irradiation. Throughout its history, the ICGFI made great achievements in helping to generate research to establish the safety and wholesomeness of irradiated foods, in promoting its effectiveness for the treatment of a wide range of food products for various technical purposes, and in successfully collaborating with the Joint FAO/WHO Codex Alimentarius Commission (Codex) and the International Plant Protection Convention (IPPC) in the finalization of international standards related to irradiation. In view of these notable achievements, the 20th ICGFI meeting (October 2003) decided that there was no further justification for the continuation of the Consultative Group beyond the expiration of its mandate in May 2004, and the body was disbanded.

Further activities related to the application of irradiation for sanitary and phytosanitary purposes continue to be carried out by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and, where appropriate, in collaboration with WHO, Codex, the IPPC and other international organizations. Examples of such activities include the sponsoring of visiting scientists, the convening of ad hoc groups of experts to provide independent and authoritative advice, and research projects supported through the FAO/IAEA technical cooperation program and other assistance programs of the agencies involved.

Conclusions of the FAO/WHO/IAEA Study Group on High-Dose Irradiation: Wholesomeness of Food Irradiated with Doses above 10 kGy (WHO, 1999)

The FAO/WHO/IAEA Study Group on High-Dose Irradiation: Wholesomeness of Food Irradiated with Doses above 10 KGy, met in Geneva, Switzerland, from 15-20 September 1997. The Study Group convened to assess the safety and nutritional adequacy of food irradiated to doses above 10 kGy. The Study Group was formed in response to the technological need for average doses higher than 10 kGy to ensure that food items, particularly meat and poultry, are rendered consistently free of pathogens. The Study Group examined other technological objectives of high-dose irradiation, including the decontamination of low-moisture products, such as spices, herbs, and dried vegetables, the preparation of sterilized meals or meal components for hospitalized patients, and the production of shelf-stable hygienic products that reduce the need for refrigeration and frozen storage and can thus facilitate safe food distribution under tropical and subtropical conditions.

The Study Group examined over four decades of research in order to address the complete range of questions raised by high-dose food irradiation. In reviewing this vast body of evidence, the experts also identified several conditions and procedures that constitute good irradiation practices for specific applications as well as principles of risk assessment related to the technology.

The Study Group report provides a brief history of food irradiation, its regulatory control, and the rationale for the upper limit of 10 kGy established by WHO in 1980. The importance of food irradiation as a public health technology is also briefly discussed. The review of radiation chemistry included studies of the chemical changes in foods and food constituents detected after high-dose irradiation, giving particular attention to the complex physical and physicochemical processes observed in muscle foods. The experts also considered evidence that foods of similar composition show similar chemical and microbiological responses when similarly irradiated, thus supporting the validity of granting broadly-based generic approvals of high-dose irradiated foods.

A review of nutritional considerations considered findings from numerous studies of the effects of high-dose irradiation on macro- and micronutrients. Apart from confirming the commonality and predictability of radiation effects, these studies supported the conclusion that irradiated foods are, from a nutritional viewpoint, substantially equivalent or superior to thermally sterilized foods.

Microbiological considerations were also addressed, including a review of the effects of irradiation on microorganisms and the factors influencing their radiation resistance. Studies evaluated cover vegetative bacterial cells, animal parasites, yeasts, mould propagules, bacterial spores, viruses, and preformed microbial toxins. On the basis of this exhaustive review, the report concludes that high-dose irradiation is no different from thermal processing in producing shelf-stable, microbiologically safe foods.

The review of toxicological safety examined findings from a considerable number of animal feeding studies and clinical studies using human volunteers. Animal investigations included carcinogenicity bioassays and multigeneration reproductive toxicity evaluations. These studies supported the conclusion that irradiated foods using a variety of sources under a variety of conditions are toxicologically safe for human consumption.

The Study Group addressed the important role packaging plays in facilitating irradiation processing, in protecting irradiated food from recontamination, and in maintaining the quality of the food. Although studies of flexible packaging are stressed, the report also assesses the suitability of all available packaging materials for use in high-dose applications, and recommends the best candidate materials and processes for the development of future generations of packaging for radiation-sterilized food. The Study Group report also considers the processing and environmental conditions and control procedures essential for ensuring that a food product is sterilized within the targeted dose range.

On the basis of the extensive scientific evidence reviewed, the Study Group concluded that food irradiated to any dose appropriate to achieve the intended technological objective is both safe to consume and nutritionally adequate. The experts further concluded that no upper dose limit need be imposed, and that irradiated foods are deemed wholesome throughout the technologically useful dose range below and above 10 kGy.

Activities of the Joint FAO/WHO Codex Alimentarius Commission

The Joint FAO/WHO Codex Alimentarius Commission has been involved in the elaboration of standards, codes of practice and other recommendations related to food irradiation for many years. The Codex Committee on Food Additives and Contaminants, Codex Committee on Food Labelling and the Codex Committee on Methods of Analysis and Sampling have all been involved in food irradiation. Currently, the Codex Committee on Food Hygiene is responsible for food irradiation.

Many of these Codex texts were elaborated in collaboration with the Joint FAO/IAEA/WHO ICGFI, whose Secretariat was located in the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Food and Environmental Protection Subprogramme, of the International Atomic Energy Agency.

Codex General Standard for Irradiated Foods

The Codex General Standard for Irradiated Foods (CODEX STAN 106-1983) was first adopted by the 13th Session of the Codex Alimentarius Commission in 1979 and was subsequently revised and adopted at the 15th Session of the Commission in 1983. The General Standard was subsequently

discussed at the 31st (1999), 32nd (2000), 33rd (2001), 34th (2002) and 35th (2003) Sessions of the Codex Committee on Food Additives and Contaminants[17].

The 35th Session (2003) of the Codex Committee on Food Additives and Contaminants noted the conclusions of the Joint FAO/WHO/IAEA Study Group on Food Irradiation that food irradiated with doses above 10 kGy was both safe and nutritionally adequate (see above). On the basis of this conclusion, and in consideration that the previous Standard stated that "*the overall average dose absorbed by a food subjected to radiation processing should not exceed 10 kGy*", the Committee reached a compromise solution and agreed to remove this limitation by defining a more practically applicable statement on dose limitation, as follows:

"*2.2 Absorbed Dose*

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 draft revised Standard was adopted by the 26th Session of the Codex Alimentarius Commission as a final Codex text[18] in July 2003.

Recommended International Code of Practice for Radiation Processing of Food

The Recommended International Code of Practice for the Operation of Irradiation Facilities Used for the Treatment of Foods (CAC/RCP 19-1979; Rev. 1-1983) was first adopted by the 13th Session of the Codex Alimentarius Commission in 1979 and was subsequently revised and adopted at the 15th Session of the Commission in 1983.

The Code of Practice was subsequently discussed at the 32nd (2000), 33rd (2001) and 34th (2002) Sessions of the Codex Committee on Food Additives and Contaminants. On the basis of these discussions, the 26th Session of the Codex Alimentarius Commission adopted the newly named Recommended International Code of Practice for Radiation Processing of Food[19] as a final Codex text in July 2003.

Codex General Standard for the Labelling of Pre-packaged Foods

The Codex General Standard for the Labelling of Pre-packaged Foods (CODEX STAN 1-1985; Rev. 1-1991)[20] was first adopted by the 14th Session of the Codex Alimentarius Commission in 1981 and was subsequently revised by the 16th (1985) and 19th (1991) Sessions and amended by the 23rd (1999), 24th (2001), 26th (2003) and 28th (2005) Sessions of the Commission.

Section 5.2 (Irradiated Foods) of the Codex General Standard for the Labelling of Pre-packaged Foods stipulates the labelling provisions for irradiated foods.

[17] Reports of the Joint FAO/WHO Codex Alimentarius Commission and its subsidiary bodies are publicly available at http://www.codexalimentarius.net/web/archives.jsp?lang=en

[18] The Codex General Standard for Irradiated Foods (CODEX STAN 106-1983, Rev 1-2003) is publicly available at http://www.codexalimentarius.net/web/more_info.jsp?id_sta=16.

[19] The Recommended International Code of Practice for Radiation Processing of Food (CAC/RCP 19-1979, Rev 2-2003) is publicly available at http://www.codexalimentarius.net/web/more_info.jsp?id_sta=18.

[20] The Codex General Standard for the Labelling of Pre-packaged Foods (CODEX STAN 1-1985; Rev. 1-1991) is publicly available at http://www.codexalimentarius.net/web/more_info.jsp?id_sta=32

General Codex Methods for the Detection of Irradiated Food

The Codex General Methods for the Detection of Irradiated Foods (CODEX STAN 231-2001)[21] were adopted by the 24th Session (July 2001) of the Codex Alimentarius Commission.

REFERENCES OF APPENDIX B

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[21] Codex General Methods for the Detection of Irradiated Foods (CODEX STAN 231-2001) is publicly available at http://www.codexalimentarius.net/web/more_info.jsp?id_sta=377

APPENDIX C – DETAILED INFORMATION ON THE DIFFERENT FOOD CLASSES/COMMODITIES

This appendix gives an overview of the recent publications concerning the efficacy of food irradiation to reduce food-borne pathogens for the food classes and food commodities concerned by the previous SCF Reports/Opinions. It supports Chapter 5 of the main body of the Opinion by providing, when available, more detailed information but does not address other aspects of the Opinion and should not be considered in isolation. Not all the food classes/commodities included in the mandate and in Chapter 5 of the main body of the Opinion are considered within this appendix, because for some of them no additional information is considered to be relevant compared to what was reported.

This appendix does not consider the chemical safety of irradiation of the various food classes/commodities, since this will be addressed in a separate Opinion by the EFSA CEF Panel (EFSA Panel on Food contact materials, enzymes, flavourings and processing aids (CEF), 2011).

For each food class/commodity, the following aspects will be discussed, wherever appropriate:

- the objectives and limitations of the use of irradiation;
- the relevant hazards (i.e. the food-borne pathogens of concerns for the specific food class/commodity);
- new data on the efficacy of irradiation to reduce these hazards;
- comparison or association of irradiation with alternative preservation methods;
- possible microbiological concerns with the use of irradiation for the specific food class/commodity.

1. Fruits

1.1. Objectives

In 1986, the Scientific Committee for Foods (SCF, 1986) recommended that fruits could be irradiated at doses up to 2 kGy. No rationale for this recommended dose was given in this SCF report.

Irradiation of fruits can be used for a large range of purposes (O'Beirne, 1989; Thomas, 1986a, b, c; Thomas, 1988): to delay ripening (Wani et al., 2008), such as to control spoilage bacteria and fungi, to kill insect and pest of quarantine relevance and to inactivate food-borne pathogens (Delincée and Bognar, 1992; Hasegawa and Moy, 1973; Kiss et al., 1974). Irradiation of fruits has been investigated not only on fresh, raw fruits, but also on fruit juices (Alighourchi et al., 2008; Buchanan et al., 1998; Gabriel and Nakano, 2009; Lee et al., 2009; Niemira et al., 2003; Niemira and Lonczynski, 2006; Wang et al., 2004) and minimally processed, ready-to-eat fruits, e.g. fresh-cut fruits and fresh fruit salads (Fan et al., 2006; IAEA 2006; Prakash and Foley, 2004). The use of irradiation was also investigated on dried fruits and nuts (Ic et al., 2007).

1.1.1. Limitations to the use of food irradiation

The irradiation dose that can be applied on fruits is limited by some detrimental consequences on quality attributes (Thomas, 1986a, b, c; Thomas, 1988). For instance, irradiation of fresh fruits led to a reduction in firmness (Jeong-Ok et al., 2008), to the loss of some vitamins (Moreno et al., 2008; SCF, 1986), or in the case of fresh juices to off-odours (Wang et al., 2006). Most studies concluded that the maximum doses which can be applied on fruits range between 1and 2 kGy. However these maximum values depend on the type of fruits, and might change with new, more resistant cultivars.

Treatments such as calcium chloride could for instance reduce the impact of irradiation on fruit firmness (Prakash et al. 2007a). The maximum doses also depend on the type of fruit product (e.g. applied on fresh versus frozen fruit juice (Niemira et al., 2003)), and the type of fruit. For instance, 14 kGy were proposed for Lycium fruit (a traditional Chinese medicine), and according to the authors, at such doses the only detrimental effect was loss in vitamin C (Wen et al., 2006). Some effect of irradiation, deleterious on fresh fruits, could be positive in another context. For instance, the impact of irradiation on fruit cell walls is a problem for the storage of fresh fruits but can be an advantage for juice extraction (Kiss et al., 1974). In addition, changes caused by irradiation on fruit juices are in many respects similar to those caused by heat treatments (SCF, 1986). Therefore these limits in irradiation doses mostly come from quality or technological considerations and are not linked to microbial safety concerns.

1.2. Hazard identification

1.2.1. Microbiological agents to be considered

Food-borne outbreaks linked to the consumption of fruits and fruit products have been mostly caused by *Salmonella*, pathogenic *E. coli*, food-borne viruses (e.g. hepatitis A, norovirus), and parasites (e.g. *Cyclospora* and *Cryptosporidium parvum*) (FAO/WHO, 2008). Other food-borne pathogens have not been reported as a cause of diseases linked to the consumption of fruits, but may contaminate fruits and fruit products and represent a hazard. For instance, melon consumed in catering establishments was associated to sporadic cases of listeriosis in the US (Varma et al., 2007). Mycotoxins producing fungi are a hazard for some dry fruits and nuts.

1.2.2. Comparison of the situation with the past

Since the 1986 SCF report, several food-borne outbreaks have been linked to the contamination of fruits by food-borne pathogens (FAO/WHO, 2008). In the EU, fruit berries and juices and products thereof still represent a very minor part the total reported food-borne outbreaks (2 outbreaks out of 2,025 verified food-borne outbreaks in the EU in 2007 (EFSA, 2009)). Outbreaks linked to fresh fruits or juices have been of concern mostly in the US where fruits and nuts represented in 2006 16% of cases from outbreaks linked to a single food vehicle (CDC, 2009). Tomatoes and melon have regularly been implicated in several *Salmonella* outbreaks in the US, some involving several hundred cases (CDC, 2007). In Europe, berries have been the vehicle of hepatitis A virus outbreaks (Hjertqvist et al., 2006). Strawberries transmitted *Giardia* and *Cyclospora* infections in the US (CDC, 1996). Unpasteurised orange juice and unpasteurized apple cider caused *Salmonella* and *E. coli* O157 outbreaks in the US (Vojdani et al., 2008). Dried fruits such as almonds have also been identified, albeit more rarely, as the source of *Salmonella* outbreaks in the US and in Europe (CDC, 2004; Ledet Muller et al., 2007). In conclusion, several fruits commodities fresh, dried or minimally processed, with low or close to neutral pH, have caused food-borne outbreaks due to bacteria, viruses and parasites. *Salmonella* have been the most frequent, but not the only, pathogen implicated.

The increase, since 1986 (WHO/FAO, 2008), of the contribution of fruits to food-borne outbreaks for which the food vehicle has been identified, particularly in the US, could be the result of an improved surveillance and reporting system. A larger diversity of countries from which fruits are imported and the development of minimally processed products might also have been important risk factors. For instance, a large *Salmonella* outbreak in the US was linked to tomato and peppers imported from Mexico (CDC, 2008), berries which transmitted parasites in the US were presumably imported from Guatemala (CDC, 1996), and some *Salmonella* outbreaks were linked to unpasteurized fruit juices (Vojdani et al., 2008).

1.3. New efficacy data available in scientific literature

Inactivation of food-borne pathogens in fruits and fruit juices was tested on inoculated pathogen populations. D_{10} values for various *Salmonella* strains in orange juice (Niemira and Lonczynski, 2006) were between 0.6 and 0.8 kGy. On fresh cantaloupe, D_{10} values were around 0.4 kGy for *Salmonella* and *E. coli* and around 1.1 kGy for *L. monocytogenes* (Rodriguez et al., 2006). However, lower D_{10} values (around 0.2 kGy) were reported for *L. monocytogenes* inoculated on the surface of whole, packaged, tomatoes (Todoriki et al., 2009). D_{10} values of the same order of magnitude, in the range between 0.25 and 0.35 kGy, were found for *E. coli* O157/H7 in apple cider (Wang et al., 2004), and 2.5 kGy achieved a 5 $\log_{10}$ reduction of the pathogen. On whole or diced tomatoes, *Salmonella* serovars had D_{10} values between 0.3 and 0.4 kGy (Prakash et al. 2007a, b).

A lower activity of irradiation against various *Salmonella* serovars was recorded in frozen orange juice concentrates, 2 kGy permitted a reduction of only 1.3 to 2.2 $\log_{10}$ (Niemira et al., 2003). Hepatitis A virus inoculated on strawberries was fairly resistant to irradiation, with a D_{10} value of 2.97 kGy (Bidawid et al., 2000). The pathogenic bacteria were more resistant to irradiation in the real fruit products compared to model foods or buffers (Niemira and Lonczynski, 2006; Rodriguez et al., 2006).

1.4. Irradiation and alternative preservation methods / technologies

1.4.1. Comparison with alternative preservation methods / technologies

The microbial safety of fruits is mostly achieved by prevention of contamination with human pathogens, which mostly occurs through handling and through contact with untreated waste water or animal effluents (SCF, 2002).

Treatments which can be used to inactivate pathogens depend on the type of fruit products. For fresh fruits and fresh cut fruit products, treatments, if any, are applied for surface decontamination, either using chemical solutions (such as chlorine) or heat treatments. In this latter case, the intensity of the heat treatment is limited by the need to avoid damages of the fruit tissues. Depending on the fruit surface morphology, reduction of food-borne pathogens can be limited to less than 1 $\log_{10}$ or reach approximately 2 $\log_{10}$ units. In addition, bacteria might have been internalized in the fruit tissues, for instance in the stem scar of tomato fruits, and in such cases surface decontamination is inefficient (WHO, 1998).

In the case of minimally processed fruits, surface decontamination is applied before processing and does not prevent potential re-contamination during processing. In contrast, irradiation could be applied to the product in its final package.

For fruit juices, pathogens can be inactivated by a wider range of treatments. In cases that heat is avoided to present fresh, unpasteurized, juices to consumers, high pressure treatments have been proposed (Pathanibul et al., 2009).

1.4.2. Association with other preservation methods / technologies

Irradiation was tested in combination with other treatments: low dose of irradiation (0.5 kGy) in combination with mild heat treatment on fresh-cut cantaloupe (Fan et al., 2006). Freezing orange juice concentrates after irradiation added 1.2 $\log_{10}$ reduction of *Salmonella* populations compared to irradiation alone (Niemira et al., 2003).

1.5. Possible microbiological concerns with the use of food irradiation

Irradiation of fruits would cause some concern if it could mask non hygienic practices during production, harvest and storage. For unprocessed fruits, as explained above, doses above 1-2 kGy frequently have detrimental effect on fruit quality, thus limiting the possibility to use irradiation to mask hygiene failure.

Concern would also occur if irradiation could promote survival or growth of food-borne pathogens, for instance by extending shelf-life or by reduction of a competitive microflora. Globally, bacterial, non spore forming, food-borne pathogens as *Salmonella* or *E. coli* O157, are not more resistant to irradiation than the spoilage or indigenous microflora of fruits. For instance, D_{10} values of the indigenous fungal microflora were around 1-1.6 kGy (Ic et al., 2007). Treatments to inactivate the spoilage microflora of fruits would therefore also inactivate bacterial human pathogens. In addition, many fruits and fruit products are too acid to permit growth of bacterial human pathogens (except, for instance, melon). In contrast, as stated above, virus such as hepatitis A were more resistant. Irradiation treatments aiming at delaying ripening of fruits to extend their shelf-life, or at killing insects, might be too low to have a significant impact on human pathogens.

2. Vegetables

2.1. Objectives

In 1986, the Scientific Committee for Foods (SCF, 1986) recommended that vegetables could be irradiated at doses up to 1 kGy. No rationale for this recommended dose was given in this SCF report. In particular, it is not clear why the recommended dose for vegetables was different from that for fruits.

Objectives of irradiation of vegetables have mostly been delay or inhibition of physiological evolution (e.g. sprouting of onion), shelf-life extension and inactivation of food-borne pathogens (O'Beirne, 1989). Irradiation has been investigated on whole, raw vegetables, but recent research has mostly been focussed on pathogen inactivation in minimally processed, ready-to-eat vegetables products and sprouted seeds.

2.1.1. Limitations to the use of food irradiation

Irradiation has the same detrimental effect on vegetables as for fruits, with induction of softening, off-odours and loss in vitamin C. The range of doses tested were similar, usually not exceeding 2-2.5 kGy (Chaudry et al., 2004; Khattak et al., 2005; Rajkowski et al., 2003; Ramamurthy et al., 2004). For fresh cut vegetables, 1 kGy treatment did not cause vitamin C loss higher than non irradiated samples over shelf-life (Farkas et al., 2003). However, for some products, such as lettuce leaves, doses should not exceed 0.5 kGy to avoid softening (Niemira et al., 2002). Some salad vegetables could nevertheless withstand higher doses (up to 4 kGy) without noticeable quality loss (Nunes et al., 2008). Higher treatments, 3-5 kGy, were proposed for powdered, dehydrated vegetables (Dong-Ho et al., 2002).

2.2. Hazard identification

2.2.1. Microbiological agents to be considered

Food-borne outbreaks linked to the consumption of fresh vegetables were mostly caused by *Salmonella*, pathogenic *E. coli*, *Shigella*, *Listeria monocytogenes*, pathogenic *Yersinia*, food-borne

viruses (e.g. hepatitis A, norovirus) and some parasites (FAO/WHO, 2008). Pathogenic spore-forming bacteria (e.g. *Clostridium botulinum)* are mostly a hazard for heat processed vegetables.

2.2.2. Comparison of the situation with the past

Reports of food-borne outbreaks linked to vegetables have increased since the publication of the 1986 SCF report, as observed for fruits (FAO/WHO, 2008). For instance, fresh produce have been the main cause of food-borne outbreak in the US: their contribution has increased from 4.3% of food-borne outbreaks in 1996 to 17% in 2006 for leafy vegetables (CDC, 2009). In the EU, fresh produce were the second cause of *Salmonella* outbreaks, expressed in numbers of cases, in 2004 (EFSA, 2006). However, in the EU, vegetables and juices and other products still represent a minor part of verified food-borne outbreaks (0.79% in 2007) (EFSA, 2009). Leafy vegetables used in salads and sprouted seeds have been the most frequently implicated vegetables (FAO/WHO, 2008).

Several outbreaks were linked to imported products (Friesema et al., 2008; Takkinen et al. 2005), some involving several countries, sometime as distant as Australia (Stafford et al., 2007). Most outbreaks concerned products of which the consumption has presumably increased since 1986 in EU, such as pre-packed vegetables or ready-to-eat pre-cut salads (e.g. in Englands and Wales, prepared salads accounted for 4% of foodborne outbreaks from 1992 to 2006 (Little and Gillespie, 2008)).

2.3. New efficacy data available in scientific literature

Inactivation of food-borne pathogens by irradiation was tested after inoculation of various kinds of vegetables and vegetable products. On various types of lettuce and leafy greens, D_{10} values for *E. coli* O157 ranged from 0.3 to 0.45 kGy (Niemira, 2008) and from 0.15 to 0.20 kGy (Mahmoud, 2010; Trigo et al., 2009). On iceberg lettuce, *Shigella flexneri* had similar D_{10} values than *Salmonella*, around 0.2 kGy. On arugula minimally processed salads, D_{10} values for *Salmonella* were in the range between 0.15 and 0.2 kGy and those for *L. monocytogenes* were between 0.3 and 0.5 kGy (Nunes et al., 2008). Similar D_{10} values were found for *L. monocytogenes* and *Salmonella* on peeled mini-carrots, minimally processed cucumbers and sprouted seeds (Caillet et al., 2006b; Dhokane et al., 2006; Saroj et al., 2006). Lower D_{10} values, around 0.16-0.2 kGy, were reported for several strains of *L. monocytogenes* on chopped romaine lettuce (Mintier and Foley, 2006) and iceberg lettuce (Mahmoud, 2010). It should be noted that these D_{10} values for *L. monocytogenes* were markedly lower than the 1 kGy D_{10} value reported for cantaloupe (Rodriguez et al., 2006). On dried seeds used for sprouts production, D_{10} values of *Salmonella* Typhimurim were around 0.5 kGy (4 $\log_{10}$ reduction achieved by 2 kGy) (Saroj et al., 2007).

Irradiation treatment of 1 kGy, low enough to preserve the sensory acceptability, resulted in the elimination of *L. monocytogenes* (around 4 to 5 $\log_{10}$ reduction) inoculated on shredded carrots, various fresh vegetables and sprouts (Bari et al., 2005; Farkas et al., 2003).

Significant differences in D_{10} values for the same pathogen strain inoculated on different vegetables were recorded. For instance, variations of 50% for the D_{10} values of *E. coli* O157 were found among various varieties of lettuce inoculated and treated under the same conditions (Niemira, 2008).

As found on fruits, hepatitis A virus on vegetables was more resistant than vegetative bacterial cells with D_{10} values around 2.7 and 3 kGy (Bidawid et al., 2000).

2.4. Irradiation and alternative preservation methods / technologies

2.4.1. Comparison with alternative preservation methods / technologies

The microbial safety of vegetables is mostly achieved by prevention of contamination with human pathogens. Food-borne zoonotic pathogens are very rarely detected in fresh vegetables (Little and Gillespie, 2008). In the EU, most surveys did not find *Salmonella* in fresh produce (EFSA, 2007a, 2009). From surveys reporting the detection of *Salmonella*, the microorganism was present in less than 1% of samples in 2006 (EFSA, 2007a) and in 2.3% of samples in one in 2007 (EFSA, 2009). The presence of Salmonella usually results from contact with untreated waste water or animal effluents (SCF, 2002; FAO/WHO, 2008). Wild-life was also identified as a source of contamination (Sagoo et al., 2003). *L. monocytogenes* has been relatively more frequently detected on fresh vegetables (Crepet et al., 2007) with around 1% positive samples in most surveys but some surveys detecting up to 5-11% positive samples (EFSA, 2007a, 2009). In addition *L. monocytogenes* has not been detected in numbers higher than 100 CFU/g in fresh produce.

Reduction of food-borne pathogen populations that could be present on vegetables is usually done by surface decontamination using chlorine solution. This permits a reduction usually equal or lower than 2 $\log_{10}$ and is not efficient whenever microorganisms have been infiltrated inside the vegetable tissues, for instance during immersion in water colder than the vegetables (WHO, 1998). The studies cited above show that for many vegetables and sprouts, irradiation can permit higher reductions of bacterial pathogens, without causing noticeable sensory damage to the vegetable. In addition, irradiation can be applied on products in the final package and is active against internalized pathogens (Niemira, 2008; Nthenge et al., 2007).

2.4.2. Association with other preservation methods / technologies

On vegetables and sprouted seeds, the effect of irradiation could be completed by washing with water (Rajkowski and Fan, 2008). The effect of irradiation was increased by packaging vegetables in atmospheres enriched with carbon dioxide or containing essential oils (Caillet et al., 2006a, b). Irradiation and chorine washing seemed to have a synergistic effect on alfalfa seeds (Thayer et al., 2006). Irradiation reduced the ability of the surviving fraction of the population of *L. monocytogenes* to grow at refrigeration temperatures on pre-cut vegetables (Farkas et al., 2003).

2.5. Possible microbiological concerns with the use of food irradiation

The possible concerns of using irradiation on vegetables are the same as for fruits and will not be repeated here. A difference between vegetables and fruits is that most vegetable tissues have a neutral pH, and most pathogenic bacteria can grow on packaged, minimally processed vegetables (Nguyen-the and Carlin, 2000). However, it should be noted that in none of the studies cited above irradiation permitted extension of shelf-life without reducing the population of vegetative pathogenic bacteria. In contrast, currently applied technologies, such as packaging with carbon dioxide atmospheres, can reduce growth of the spoilage microflora of pre-cut vegetables, without any significant reduction of the growth of pathogenic bacteria (Carlin et al., 1996).

3. Spices and condiments

3.1. Objectives

Spices and herbs, in their natural state, contain a large number of microorganisms capable of causing spoilage of foods to which they are added (Farkas, 2000). A wide variety of spore-forming and non spore-forming pathogenic bacteria and moulds may be present. Although spices may not be suitable

substrates for the growth of pathogenic bacteria, even occasional *Salmonella* contamination is a reality. Irradiation is a recognized and feasible method both for disinfestation of whole and ground spices (Padwal-Desai et al., 1987) and reducing the microbial load of spices and condiments with minimal effects on their sensory properties (Farkas, 1988; IAEA, 1992).

3.2. Hazard identification

3.2.1. Microbiological agents to be considered

The microbiology of spices and herbs has been reviewed in detail (Farkas, 2000; ICMSF, 1998). Among the spore-forming bacteria, *Bacillus cereus* and *Clostridium perfringens*, which are capable of causing gastroenteritis when ingested in large numbers, are frequently found in spices, but usually in low numbers. In extreme cases, however, *Bacillus cereus* counts up to 10^5 CFU/g have been found (Giffel et al., 1996). Several other *Bacillus* spp. that are opportunistic pathogens are more frequently isolated from spices. Since bacterial spores may survive cooking temperatures, ingredients harbouring these spores must be considered as a potential health hazard (Farkas, 2001).

Salmonellae has been found, albeit infrequently, in a variety of spices (Bruchmann, 1995). However, their presence is of special concern when spices are used in foods that are consumed raw, or when the spices are added to foods after cooking. Depending on the temperature and water activity, salmonellae can remain viable in spices and condiments for significant storage periods (Ristori et al., 2007). Peppers have been implicated in salmonellosis outbreaks in Canada, Norway and Sweden (Gustavsen and Breen, 1984; Persson, 1988; Severs, 1974). In 1993, a nationwide outbreak of salmonellosis occurred in Germany following ingestion of paprika and paprika-powdered potato chips contaminated with a great variety of *Salmonella* serovars (Lehmacher et al., 1995). This largest documented outbreak (of the estimated 1,000 cases), due to contaminated spices proved that extremely low numbers of salmonellae adapted to the dry state were able to cause illness. In 2009, due to a multi-state *Salmonella* Rissen outbreak in the United States numerous spice products were recalled, and another multi-state outbreak of *Salmonella* Montevideo infection by salami products was confirmed to be related to their ingredients, black and red peppers, as a source of contamination[22].

Mould counts of spices and herbs may reach the 10^5 propagules per gram level, and a relatively high incidence of toxigenic mould has also been found (McKee, 1995). Climate change and global warming (IPCC, 2007) is expected to increase the problem of mycological safety of spices, important commodities of the international trade.

3.2.2. Comparison of the situation with the past

The markets for spices and herbs in industrialized countries are increasing with the growing popularity of ethnic foods. One has also to bear in mind that even "regular" microbial contamination of dry dietary ingredients may pose a health hazard to severely immuno-compromised individuals.

3.3. New efficacy data available in scientific literature

Recent publications on irradiation of spices and condiments did not produce entirely new aspects or type of information as compared with the extensive former literature, and they proved the technological feasibility of radiation processing of additional items (Farkas, 1998). D_{10} values described in recent publications are in line with the general ones indicated in Chapter 3 of the main body of the present Opinion.

[22] http://cdc.gov/salmonella/montevideo/index.html

3.4. Irradiation and alternative preservation methods / technologies

3.4.1. Comparison with alternative preservation methods / technologies

Fumigation with ethylene oxide was routinely practiced for many years but has been banned in most countries since it was recognized as a potential carcinogen. Satisfactory decontamination of certain spices by various heat treatments is possible but many of them suffer serious loss of quality (flavour, colour) from any sort of heat processing. For some dry vegetable seasonings like onion powder, irradiation is the only satisfactory decontamination treatment. Technical and economic advantages of irradiation over other methods of processing spices have been summarized by Modak (1993). The landmark directives of the European Union in February 1999 allowed irradiation of spices and dry aromatic herbs with up to 10 kGy dose levels, beginning in 2000. Prior and after this date, clearances of many countries worldwide have been granted for spices and condiments. Irradiation of spices is also a reality in many third countries, including the United States, China, Australia and most of the important spice producing countries such as India, Vietnam, Indonesia, Republic of Korea, Thailand, Mexico, Brazil and South Africa (IAEA, 2009; Loaharanu, 1994). In the USA alone, about 80,000 metric tons of spices were irradiated each year between 1986 and 2006, approximately one third of the commercial spices consumed annually in that country.

4. Fish and shellfish

4.1. Objectives

In 1986, the Scientific Committee for Foods (SCF, 1986) recommended that fish and shellfish could be irradiated at doses up to 3 kGy.

Irradiation of fish and shellfish is intended to extend shelf-life, reduce pathogen load and inactivate parasites. Another application is the disinfestation of dried fish and smoked fish to prevent deterioration due to insect damage (Boisot and Gauzit, 1966; Venugopal, 2005). Irradiation has been applied to fresh, frozen as well as dried fish, fish products, and shellfish.

4.1.1. Limitations to the use of food irradiation

Content of fat in the fish is a limiting factor, since irradiation can impart undesirable organoleptic attributes to high-fat fish. There are a series of changes such as radiation-induced oxidative rancidity and other sensory changes (fading of red colour of gills, greenish colour in tuna and salmon, textural changes with drip). Sensory changes depend on the type of fish and can be reduced by vacuum-packaging. However vacumm-packing must be associated to refrigeration or freezing to prevent the risk of *Clostridium botulinum*.

Fish and shellfish are products with a short shelf-life since rapid microbial spoilage can occur shortly after harvest. Irradiation has to be applied soon after harvest to prevent microbial growth which may render the product inappropriate for processing or consumption.

4.2. Hazard identification

4.2.1. Microbiological agents to be considered

Biological agents responsible for food-borne diseases are commonly found in fish and shellfish and include agents such as pathogenic *Vibrios*, *Salmonella* spp., pathogenic *Escherichia coli*, *Yersinia enterocolitica*, *Clostridium botulinum*, *Shigella*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, *Klebsiella*, parasites (*Anisakis simplex* and other helminths), viruses (hepatitis A

virus, norovirus, enteric viruses). Disease caused by pathogenic Vibrios, *Listeria monocytogenes,* viruses and parasites is related to consumption of raw products.

Most of the recent work on the use of irradiation in fish and shellfish products has concentrated on reduction of pathogens such as *Listeria monocytogenes, E. coli* O157:H7, as well as more classical pathogens such as *Staphylococcus, Salmonella, Vibrio* and Clostridia.

4.2.2. Comparison of the situation with the past

In recent years safety risks associated with the consumption of fish and shellfish have been highlighted, especially in products eaten raw or those originating from tropical or subtropical sites. According to the Community Summary Report (EFSA, 2009), fish and fish products were responsible for 6.4% of verified outbreaks in the EU in 2007, including those caused by biotoxins and amines. A number of reports dealing with *Vibrio, Salmonella* and viruses in fish and shellfish (molluscs) have been published (Dalsgaard, 1998; Igbinosa and Okoh, 2008; Lees, 2000; Lyytikinen et al., 2006; Norhana et al., 2010; Robertson, 2007; Su and Chengchu, 2007).

The increasing trend in raw fish consumption (sushi, sashimi, salmon, etc.) has been identified as a risk. Disease caused by pathogenic *Vibrio*, *Listeria monocytogenes* and viruses is related to consumption of raw products. Allergies and parasitism have been associated with nematodes (*Anisakis simplex*), which have high prevalence in wild fish from certain fishing grounds. Pathogens related to aquaculture products from tropical and subtropical areas and products such as bivalves should be also considered. Trematodes (*Chlonorchis, Opistorchis,* etc.) associated with fish and fish products have high prevalence in South-East Asia. An increase in prevalence for some pathogens (*Vibrio* spp.) as a result of extremely warm seasons has already been detected in European coastal areas (Caburlotto et al., 2008; Gras-Rouzet et al., 1996; Hoi et al., 1998). Development of blooms (red tides), which could eventually lead to the presence of toxic microalgae in fish, has also a strong dependence on environmental conditions.

4.3. New efficacy data available in scientific literature

Publications dealing with the effect of irradiation (γ-irradiation, X rays, or electron beam irradiation) on microbial populations in fish describe the inactivation kinetics of indigenous/inoculated pathogens or spoilage microflora, and the extrinsic/intrinsic factors influencing inactivation. Most studies obtain D_{10} values for pathogens or test different processing conditions. *Listeria monocytogenes* inactivation kinetics has been investigated in cold smoked salmon (Medina et al., 2009; Su et al., 2004), ready-to-eat, vacuum-packaged smoked mullet (Robertson et al., 2006) and trout (Savvaidis et al., 2002). *E. coli* O157:H7 has been studied in trout fillets, and D_{10} values ranged between 0.22 kGy to 0.33 kGy (depending on the a_w) (Black and Jaczynski, 2008). The hygienic quality and the evolution of pathogens (*Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp., *Escherichia coli*, *Staphylococcus aureus, Vibrio parahaemolyticus,* etc.) have been studied in dried fish Bajirak jeotkal (Song et al., 2009c), kwamegi (traditional Korean sea food) (Chawla et al., 2003; Lee et al., 2002), seafood products (dried seaweed) (Jo et al., 2005), surimi seafood (Jaczynski and Park, 2003), needle fish and crucian (Cui et al., 2000), low (*Coillia dussumieri*) and high fat fish (*Sardinella longiceps*) (Kamat and Thomas, 1999). According to Jo et al. (2005), the D_{10} values of these organisms ranged from 0.23 to 0.62 kGy in imitation crab leg, 0.31 to 0.44 kGy in surimi gel, and 0.27 to 0.44 kGy in dried seaweed. In a study of Kamat and Thomas (1998) the D_{10} values in kGy ranged from 0.2 to 0.3, 0.15 to 0.25, 0.1 to 0.15 and 0.09 to 0.1 for *L. monocytogenes* 036, *B. cereus, S. typhimurium* and *Y. enterocolitica* F5692, respectively. Changes in microbial communities including moulds have been studied in ready-to-eat smoked sardines (*Sardinella* spp.), marinated fish (*Diplodus puntazzo*) (Nketsia-Tabiri et al., 2003), *Tilapia nilotica* (fresh, semidried and dried) (El-Mongy et al., 1996; Ghaly et al. 1998), sliced dried squid (Jongkwan et al., 2005) and ice stored blue crab (*Callinectes*

sapidus) meat products (Chen et al., 1996). D_{10} values described in recent publications are in line with the general ones indicated in Chapter 3 of the present Opinion.

The effect of irradiation on parasites has also been reported, such as for opistorchide metacercariae (Naz'mov et al., 2001) or radioresistant *Anisakis simplex* third-stage larvae in sea eel *Anago anago* (Seo et al., 2006). Third stage larvae of *Anisakis simplex* have a high degree of radioresistance and it is not possible to inactivate the parasite at the doses normally used and possibly even at doses higher than 6 kGy (Chai et al., 1991; Loaharanu and Murrell, 1994; Padovani et al., 2005; Seo et al., 2006). In contrast freezing can inactivate *Anisakis* in raw fish. The EFSA Panel on Biological Hazards (BIOHAZ) (2010) recently reported some additional information on the effect of irradiation on parasites in fish. No clear information is available about allergenicity of the dead parasite.

Several articles describe the survival and change in numbers of pathogenic *Vibrio* in oysters (*Crassostrea brasiliana, Crassostrea virginica*) (Andrews et al., 2003; de Moraes et al., 2000; Hu et al., 2005) or pathogen cocktails (*Listeria monocytogenes*, *Staphylococcus aureus*, and *Vibrio parahaemolyticus*) in salted, seasoned, and fermented oysters (Song et al., 2009a), after irradiation processes up to 3 kGy. Inactivation of other pathogens (*Salmonella* Enteritidis, *Salmonella* Infantis, and *Vibrio parahaemolyticus* (Jakabi et al., 2003) and *Cryptosporidium parvum* (Collins et al., 2005)) has also been studied.

4.4. Irradiation and alternative preservation methods / technologies

4.4.1. Comparison with alternative preservation methods / technologies

High pressure treatment (450MPa) has been proposed to reduce *L. monocytogenes* in cold-smoked salmon (Medina et al., 2009). High hydrostatic pressure is also commonly used to process bivalves and shellfish (Murchie et al., 2005).

4.4.2. Association with other preservation methods / technologies

The combined effect of γ-irradiation and trisodium phosphate (TSP) for preservation of fish fillets for the improvement of microbiological quality has been studied (Mohamed et al., 2008). Combination of 3% TSP and 2 kGy of irradiation reduced the aerobic plate count to less than 2 $\log_{10}$ cycles with satisfactory sensory and chemical attributes.

4.5. Possible microbiological concerns with the use of food irradiation

As fish products have a better quality if processed vacuum-packed, there is a concern for growth of anaerobic, irradiation-resistant bacteria such as *Clostridium botulinum*, if the products are not properly refrigerated. Other radio-resistant agents such as hepatitis A virus, parasites (*Anisakis simplex*) or marine toxins which may be present in fish or molluscs are also of concern.

5. Poultry

5.1. Objectives

Irradiation of poultry up to an overall average dose of 7 kGy was proposed by the SCF (1986). The main objectives for irradiating poultry meat are pathogen reduction and extension of shelf-life.

5.2. Limitations to the use of food irradiation

Organoleptic quality is reported to be affected above 2.5 kGy in fresh chicken and 1.5 kGy in fresh turkey (when irradiated at 5-10°C). The threshold dose for detectable "off-flavour" is at least twice as high for frozen poultry meat as it is for chilled products (Sudarmadji and Urbain, 1972).

5.3. Hazard identification

5.3.1. Microbiological agents to be considered

Based on the available information on the number of cases of food-borne illness, the food-borne pathogens of higher concern in raw poultry meat are represented by *Campylobacter* spp., *Salmonella* spp. and pathogenic *Escherichia coli*. There is the potential for many other zoonotic agents to be present in/on the raw meat, but the risk is relatively low, compared to those mentioned above.

Campylobacter infections continue to be the most frequently reported zoonotic diseases in humans across the EU. In 2008 there were 190,566 confirmed cases reported which represents a decrease of 5.0% from the previous year. This pathogen was mostly found on raw poultry meat, with an average of 30% of samples showing contamination (EFSA, 2010). *Salmonella* infections across the EU have decreased between 2003 and 2007. In 2008, 131,468 people were affected compared to 172,705 in 2003. On average 5.1% of all fresh poultry meat samples within the EU were found to be contaminated (EFSA, 2010) with these bacteria.

When considering cooked chilled and other ready-to-eat poultry meat products, the food-borne pathogens of higher concern are represented by *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus* and non-proteolytic type B *Clostridium* spores (if packaged without oxygen). Vegetative pathogens are sensitive to heat, so proper cooking will eliminate them from food. However, cross-contamination can occur post-cooking e.g. during subsequent slicing and packaging.

The pathogen of most concern in ready-to-eat meats is *Listeria monocytogenes*, especially as it can grow at refrigeration temperatures. There were 1,381 confirmed cases of listeriosis reported in the EU in 2008, compared to 1,070 in 2003 (EFSA, 2010). Although the number of cases remains relatively low, *L. monocytogenes* has a high mortality rate (20%) especially in the elderly and other vulnerable groups. Data on ready-to-eat food from broiler or other poultry meat showed that *L. monocytogenes* was detected qualitatively in samples ranging from 0% to 7.6% in 11 Member States. *L. monocytogenes* was only found in levels above 100 CFU/g in 0.1% of samples (EFSA, 2010).

Salmonella contamination in ready-to-eat chicken and turkey meat was relatively low at 0.2% and 0.6% respectively.

Toxin-producing strains of *Staphylococcus aureus* may be of concern if the cooked product is handled by humans during processing, post-cooking and stored at abuse temperatures. The lack of competition in the cooked product may allow the cells to multiply to reach sufficiently high numbers to allow toxin to be produced.

Bacterial spores are generally not present in high numbers in raw poultry meat. However, if present, they can survive the cooking process. Non-proteolytic type B *C. botulinum* strains can potentially grow, and produce toxin, in extended shelf-life cooked meats stored in the absence of oxygen.

5.3.2. Comparison of the situation with the past

Improvements in animal husbandry and the *Salmonella* control programmes in breeding flocks in Member States (mandatory since 2007 but already in place in some countries prior to this date) have

led to a decrease in the overall incidence of *Salmonella* in raw poultry. However, the changes in animal husbandry have not had the same effect in lowering the incidence of *Campylobacter* in raw poultry meat and this pathogen is likely to continue to be a major contaminant in these products.

Consumption of poultry products within the EU has increased year-on-year and this trend is expected to continue in the next 5 years (EC, 2009). In particular, there has been an increase in the range and volume of processed/ready-to-eat products and a decrease in sales of whole birds (Magdelaine et al., 2008).

The Community legislation (Regulation (EC) No 2073/2005) lays down food safety criteria for *Listeria* in ready-to-eat foods. This regulation came into force in January 2006. *L. monocytogenes* must not be present in levels above 100 CFU/g during the shelf-life of the product. In addition, products in which growth of the bacterium is possible, must not contain *L. monocytogenes* in 25g at the time they leave the production plant unless the producer can demonstrate to the competent authority that the product will not exceed 100 CFU/g limit throughout shelf-life. Producers may seek additional measures to ensure their ready-to-eat products are in compliance with the Regulation. Irradiation is one option that they may consider.

5.4. New efficacy data available in scientific literature

5.4.1. Raw poultry products

Further research has confirmed previous findings on the radiation-response of the main vegetative pathogens (*Campylobacter*, *Salmonella*, *Listeria monocytogenes*, *E. coli* O157:H7) when treated in raw poultry meat (Dion et al., 1994; Gursel and Gurakan, 1997; Patterson, 1995; Sanos et al., 2003; Thayer et al., 1995a; Thayer et al., 1998).

As expected the D_{10} values varied depending on treatment conditions. Bacteria were more resistant when treated at frozen temperatures compared to chill (Mayer-Miebach et al., 2005; Thayer and Boyd, 1993). A reduction in water activity also increased radiation resistance (Black and Jaczynski, 2008). The presence of oxygen can increase sensitivity to irradiation (Thayer and Boyd, 1999).

Based on these observations, the current position of treating poultry meat with up to 7 kGy would be sufficient to give at least a 5-$\log_{10}$ reduction of the most resistant vegetative pathogens treated in frozen meat. Treatments of about 3.5 kGy would be sufficient for chilled meats.

5.4.2. Ready-to-eat poultry products

The majority of poultry publications in recent years have focused on the effects of irradiation on the microbial quality of ready-to-eat products, reflecting the increased importance of these in the marketplace.

Post-processing contamination with *L. monocytogenes* is of most concern in ready-to-eat meats and there are a number of published studies which investigated the potential of irradiation to control the pathogen in cooked poultry products (chicken and turkey). Thayer et al. (1998) reported D_{10}-values for *L. monocytogenes* in cooked turkey nuggets as about 0.70kGy, making *L. monocytogenes* generally more radiation-resistant than *Campylobacter* and *Salmonella*.

Some authors noted that if some *L. monocytogenes* did survive the irradiation treatment (usually as a result of a high initial inoculum), the cells could grow during subsequent chill storage of the cooked meat (Foong et al., 2004; Suvang et al., 2007; Zhu et al., 2008).

The irradiation sensitivities of *E. coli* and *Staph. aureus* on poached chicken and minced chicken were determined. The D_{10} values for *E. coli* on the poached and minced chicken were 0.18 and 0.25 kGy respectively while for *Staph. aureus*, the values were 0.27 and 0.29 kGy, respectively. The sensory quality of the poached chicken was not affected by an irradiation dose of 3 kGy (Adu-Gyamfi et al., 2008).

5.5. Irradiation and alternative preservation methods / technologies

5.5.1. Comparison with alternative preservation methods / technologies

Irradiation treatment can be regarded as a Critical Control Point in the HACCP plan for both raw and cooked meats. The products are packaged before irradiating, so this would be the final decontamination step. Although the 7 kGy maximum radiation dose proposed for poultry meat will significantly reduce the numbers of any vegetative pathogen that may be present, this is not a sterilizing dose. Therefore, as with heat pasteurization, additional control measures, such as chill storage, need to be taken in order to gain the maximum shelf-life benefit. Irradiation cannot be seen as a substitute for GMP. The initial quality of the meat is still important and procedures still need to be in place to minimize post-cooking contamination e.g. during slicing and packaging before irradiation and to ensure that they are stored in appropriate chill conditions during shelf-life.

There has been a significant increase in the volume of cooked poultry meats in the market. Correctly applied, the heat treatment should be sufficient to eliminate vegetative cells as well as bacterial spores (it is recommended that ready-to-eat vacuum packaged meats are given a decontamination treatment of 90°C for 10 minutes, or equivalent, to ensure a 6D reduction in non-proteolytic *C. botulinum* spores). Therefore, these products should be microbiologically safe.

A study was conducted to evaluate the efficacy of three chemical disinfectants (calcium hypochlorite, lactic acid and hydrogen peroxide) and irradiation (2-7 kGy) in reducing the level of contamination in chicken carcasses which had been artificially contaminated with *Salmonella* Virchow (approximately $5x10^3$ CFU per sample). The number of carcasses which gave positive results for the presence of *Salmonella* decreased after chemical treatment, but the pathogen was not completely eliminated. However, in the carcasses subjected to 7 kGy, *Salmonella* was eliminated and no changes in the appearance, color or smell of the carcasses were observed (Nassar et al., 1997).

High pressure processing (HPP) is another physical treatment, like irradiation, which can deliver a "cold preservation" effect. However, unlike irradiation, there is less consumer resistance to the technology and it is used commercially to treat a range of ready-to-eat meats, including poultry.

Accelerated electrons have low penetrative capability and the depth limit for 10 MeV is only around 4 cm in high-moisture foods. Gamma rays and X-rays have greater penetrating powers and can be used to treat food, even in pallet-sized containers. Except for the differences in penetration, the effects of electromagnetic radiation and electrons are equivalent for treating food (Farkas, 2005). For this reason, gamma radiation is more suited for treating whole carcasses, but electron-beams could be used for packs of relatively thin cooked, sliced meats and other ready-to-eat products.

5.5.2. Association with other preservation methods / technologies

Combination of chemical preservatives and irradiation may help suppress the growth of surviving microorganisms during subsequent storage.

The combination of antimicrobials potassium benzoate + sodium lactate or sodium lactate + sodium diaceate in poultry meat followed by irradiation at 1.0 or 2.0 kGy were found to be beneficial in

suppressing growth of *L. monocytogenes* during storage of the meat at 4°C, without affecting sensory quality (Zhu et al., 2009). However, those treatments are not permitted in the EU at present.

Several studies have shown that irradiation can be used in combination with vacuum or modified atmosphere packaging in order to inhibit the growth of surviving microorganisms during chill storage (Romero et al., 2005; Thayer and Boyd, 1999).

High hydrostatic pressure can sensitize *Clostridium* spores to subsequent irradiation (Crawford et al., 1996).

5.6. Possible microbiological concerns with the use of food irradiation

Listeria monocytogenes is of particular concern in cooked and ready-to-eat meats, especially those exposed to contamination after thermal processing and with an extended shelf-life. It tends to be more radiation resistant than other vegetative pathogens, such as *Salmonella*, and can grow at refrigeration temperatures. There is a potential concern that pathogens which either survive the irradiation process, or are introduced onto foods after treatment, may multiply faster because of lack of microbial competition. This would be particularly relevant for cooked meats. Several studies have shown that if *L. monocytogenes* were to survive the irradiation treatment, they may be injured initially but can recover and multiply during extended storage (Suvang et al., 2007; Zhu et al., 2008). As already mentioned, irradiation cannot be seen as a substitute for GMP. Procedures still need to be in place to minimize post-cooking contamination e.g. during slicing and packaging before irradiation and to ensure that they are stored in appropriate chill conditions during shelf-life. However, one study showed that *L. monocytogenes* did not multiply faster during storage at 7°C when inoculated onto irradiated raw ground turkey (2.5 kGy treatment at 5°C) compared to nonirradiated controls. Thus, the authors concluded that irradiating ground turkey did not decrease its safety when it was contaminated following processing with *L. monocytogenes* (Thayer and Boyd, 2000).

Non-proteolytic *C. botulinum* type B may survive cooking of chicken meat and subsequent irradiation treatment up to 7 kGy, and may produce toxin in the meat if stored under anaerobic conditions. Guidelines in some EU member states suggest that if vacuum or MAP packaged meats are given a shelf-life of > 10 days at < 8°C, then a 6D reduction of psychrotrophic *C. botulinum* should be achieved e.g. through heating at 90°C for 10 minutes, or equivalent. The ability of *C. botulinum* spores to survive, grow and produce toxin in poultry meat during storage after irradiation has been studied by a few authors. The studies used mechanically deboned chicken meat and raw chicken skin inoculated with proteolytic *C. botulinum* type A and B and irradiation up to 3 kGy. Toxin production did not occur on any samples during storage, provided the storage temperature was < 10°C (Dezfulian and Bartlett, 1987; Thayer et al., 1995b).

There is a potential concern that the free radical scavenging effect of antioxidants, such as vitamin E, added to meats might reduce the antimicrobial effectiveness of irradiation treatment. A study was conducted to determine the effect of vitamin E on the growth of *L. monocytogenes* and colour stability in turkey meat following electron beam irradiation. The meat was obtained from birds fed diets containing various levels of vitamin E (up to 200 IU/kg). Irradiation at 2.0 kGy resulted in approximately 3.5 $\log_{10}$ reduction in initial numbers of the pathogen. Vitamin E treatments did not affect the survival of *L. monocytogenes*, but the irradiated meat from birds fed 100 or 200 IU/kg had better color stability during storage of the meat post treatment (Romero et al., 2005).

6. Fresh red meat

Red meat is derived from a number of animal species in different regions of the world (e.g. cattle, sheep, goat, camel, deer, buffalo, horse, pig, rabbit and exotic species). Many of those species are raised intensively (housed) and others only extensively. International trade in red meats is a very large

business and developed from initially shipping frozen whole carcasses, and subsequently to chilled carcasses, to portions of carcasses with the bones removed (primal cuts), often vacuum-packed. Red meat is converted into a wide range of meat products including cured (both raw and cooked), fermented (high acid and low acid, both raw and heated). Despite many attempts, there is no universally agreed classification of meat products, making separation of meat products into categories representative of different practices and cultures difficult and complex.

For the purpose of this Opinion, consideration will be limited to the primary product i.e. fresh red meat (as carcasses, cuts, or comminuted (ground, minced) meat), frozen fresh meat and dried fresh meat.

6.1. Objectives

Irradiation of fresh meat up to an overall average dose of 2 kGy was proposed by the SCF (1986). Similarly to poultry meat, the main objectives for irradiating fresh meat are the reduction of numbers or elimination of pathogenic microorganisms and the extension of shelf-life of fresh meat.

6.1.1. Limitations to the use of food irradiation

Irradiation of fresh meat can cause changes to the colour, odour and taste. This is seen by some as a major limitation to the use of irradiation of fresh meat. However, such changes can be reduced by modified atmosphere packaging, reducing the temperature (e.g. irradiating in the frozen state) and addition of antioxidants, as reviewed by Brewer (2009).

6.2. Hazard identification

6.2.1. Microbiological agents to be considered

Red meat has the potential to carry pathogenic organisms to consumers. Historically, the major public health problems were from zoonoses, such as bovine tuberculosis and brucellosis. Classical meat inspection has proved very effective against those zoonoses. More recently the main problems are "latent zoonoses", where pathogens occur as a reservoir in healthy animals but produce no pathological conditions or visible changes. Those pathogens can contaminate the meat, for instance during slaughtering, and subsequently enter the food chain. Hence, strict attention to good practices of slaughter hygiene in meat production is of crucial importance, because those microbiological hazards are not eliminated in the slaughtering process. The hazards (or "microorganisms of concern") associated with meat production remain largely the same in different regions of the world, excepting where zoonoses have not been controlled. However, with the worldwide implementation of veterinary controls at animal production and at the abattoir, fresh meat is rarely implicated in food-borne illness. This has been achieved by the control of diseases in live animals and the reduction of carriage of particular pathogenic bacteria and of parasites. Nevertheless, meat can carry microorganisms of concern and, if incorrectly processed and/or stored at inappropriate temperatures, can be implicated in illness.

Comprehensive reviews of the microbiology of meat and meat products, the microbiological hazards and the control measures are easily available in the scientific literature (ICMSF, 1998). Bacteria able to cause food-borne disease, and which can constitute a hazard in fresh meat and products thereof, include *Salmonella* spp. (common in pork and horsemeat, but less common in other meats), thermophilic *Campylobacter* spp. (common in pork), enterohaemorrhagic and verocytotoxigenic *Escherichia coli*, e.g. serogroup O157 (most common in cattle), some serovars of *Yersinia enterocolitica* (common in pork) and of *Listeria monocytogenes* (most common in ruminants and of concern especially because of its ability to growth in some meat products at refrigeration

temperature), *Clostridium perfringens*, *Staphylococcus aureus* and *Clostridium botulinum*. Contamination of fresh meat with parasites, such as *Trichinella* and *Toxoplasma*, are also a cause of food-borne infection. For many years salmonellae were the microorganisms of most concern. Since the early 1990s there have been several large outbreaks attributed to *Escherichia coli* (VTEC/EHEC) in a range of meats, especially beef, with fatalities and serious kidney damage in survivors (SCVPH, 2003). Cooking meat before consumption kills vegetative bacterial pathogens and parasites. However, many products, e.g. cured and fermented products, are eaten without cooking, yet have a good record of microbiological safety. In some countries ground beef is eaten raw and is sometimes mixed with raw ground pork or raw ground turkey. Cases of salmonellosis sometimes occur after consumption of raw ground meat when hygiene at the abattoir and/or subsequently has not been maintained adequately. When Good Husbandry and Veterinary Practices at the farm are followed by the application of HACCP in the abattoir and throughout manufacture of meat products, consumption of meat and meat products is considered safe with respect to pathogens listed above. When illness is traced to meat or to meat products, the reason is usually a failure of one or more processes in production, distribution, sale or most commonly a failure of temperature control during cooking, cooling or refrigerated storage.

6.3. New efficacy data available in scientific literature

Publications appeared in recent years with regard to irradiation of fresh meat confirmed the validity of the previously established principles and sensitivity of microorganisms (*Salmonella* spp., *E. coli* 0157:H7, *Yersinia* spp., *Listeria monocytogenes*) to treatment with irradiation, expressed as D_{10} values. D_{10} values identified by studies on fresh red meat for the different food-borne pathogens are in line with those described above for the same agents on poultry meat and more generally in Chapter 3 of the main body of the Opinion (Arthur et al., 2005; Badr, 2004; Bari et al., 2006; Black and Jaczynski, 2008; Gezgin and Gunes, 2007; Kiss et al., 2001; Rajkowski et al., 2006; Schilling et al., 2009; Sommers and Niemira, 2007; Sommers and Novak, 2002). Having identified the purpose of the irradiation treatment (elimination of particular pathogenic bacteria or extension of shelf-life) and the condition of the food to be irradiated (unfrozen, frozen or dried), the dose required can be calculated from knowledge of microbial resistance. Typical "pasteurizing" doses to eliminate vegetative bacterial pathogens and to extend shelf-life are of the order of 1-5 kGy unfrozen and 6-10 kGy frozen. Kijlstra and Jongert (2008) recently reported the results of studies conducted on the effects of irradiation on cysts of *Toxoplasma gondii*, confirming the high sensitivity of the parasite to irradiation (D_{10} values between 0.4 and 0.7 kGy).

Some studies compared the effect of irradiation of meat with different radiation sources. Rajkowski et al. (2006) indicated a significant difference between the D_{10} values obtained while irradiating ground pork inoculated with *Salmonella* Typhimurium DT 104 with gamma rays (0.56 to 0.62 kGy) and with e-beams (0.42 to 0.43 kGy), indicating a higher efficacy of e-beams. Similar conclusions were drawn by Miyahara and Miyahara (2002), indicating that, when treating with irradiation ground beef patties, e-beams were more effective than gamma rays in the case of *B. cereus* and slightly more effective in the case of E. coli O157:H7. In the case of other bacteria (*Clostridium perfringens*, *Listeria monocytogenes* and *Salmonella* Enteritidis) the two irradiation sources were similarly effective. Lopez-Gonzalez et al. (1999) reported lower D_{10} values for *E.coli* O157:H7 when treating ground beef patties with e-beams compared to gamma irradiation.

6.4. Irradiation and alternative preservation methods / technologies

6.4.1. Comparison with alternative preservation methods / technologies

Considerations similar to those made for poultry meat in the same section of the chapter above can be also made for fresh red meat. Alternative processing methods that can be used in fresh red meat are also similar.

Schilling et al. (2009) compared the effects of irradiation and hydrostatic pressure on the reduction of *Escherichia coli* 0157:H7 on frozen ground beef patties. The study concluded that while irradiation at 2 kGy was able to reduce the pathogen from a concentration of 10^3 CFU/g to non-detectable levels, hydrostatic pressure (300 MPa for 5 minutes at 4°C) was not able to reduce the pathogen so effectively. However, other studies demonstrated that hydrostatic pressure applied under other conditions (345 MPa for 5 minutes at 50°C) was able to reduce the same pathogen and other microogranisms by more than 6 logs (Alpas et al., 1999).

Ahmadi et al. (2006) developed a Monte Carlo simulation model in order to estimate the effect of different possible interventions on the reduction of contamination of dairy-beef quarters in Dutch slaughterhouses. Of all the single interventions applied (hot-water wash, lactic-acid rinse, trim, steam-vacuum, steam-pasteurization, hide-wash with ethanol, gamma irradiation), irradiation was the most effective treatment and the only one that could eliminate almost all the bacterial population (decreasing prevalence by over 99%), even in high contamination situations. A comparable effect was obtained only associating several other interventions (hot-water wash, lactic-acid rinse, steam-pasteurization and hide-wash with ethanol).

As indicated elsewhere in the document, e-beams have a lower penetrative capability compared to gamma rays and are therefore appropriate to treat meat when cut in relatively thin slices. Arthur et al. (2005) also investigated the use of e-beams at low dose (1 kGy) for treating beef carcasses during processing as a method to reduce surface carcass contamination, obtaining reductions of inoculated *E.coli* 0157:H7 in the order of at least 4 $\log_{10}$ CFU/cm^2.

6.4.2. Association with other preservation methods / technologies

Heat-sensitisation induced by prior low dose irradiation was investigated by Grant and Patterson (1995). Results indicated that a prior treatment at 0,8 kGy of roast beef contaminated with *Listeria monocytogenes* and *Salmonella* Typhimurium was able to induce an increased susceptibility of those bacteria when subsequently submitting the meat to a heat treatment at 60°C (both in the case of *Salmonella* and *Listeria*) and at 65° and 70°C (only in the case of *Listeria*).

More recently Chiasson et al. (2005) investigated the effect of the combination of four different atmosphere conditions (air, CO_2, MAP with 60% O_2, 30% CO_2 and 10% N_2 and vacuum), a mixture of carvacrol and tetrasodium pyrophosphate (TSP) and irradiation on the reduction of *Escherichia coli* and *Salmonella* Typhi in ground beef. The results indicated that the use of MAP had a higher effect in reducing the D_{10} values for both microorganisms compared to the other atmospheres and that the D_{10} value was further reduced by the application of carvacrol and TSP.

6.5. Possible microbiological concerns with the use of food irradiation

Considerations expressed above in relation to irradiation of poultry meat and possible concerns more generally described in Chapter 4 of the main body of the Opinion are similarly valid for fresh red meat.

7. Camembert cheeses manufactured from raw milk and cheeses in general

7.1. Objectives

In the 1990s, the use of gamma-irradiation at doses up to 2.5 kGy was requested as a means of controlling the microflora of Camembert cheese manufactured from raw milk (SCF, 1992). The SCF agreed that irradiation of this product at the indicated dose was acceptable from a health point of view. The main objectives for irradiating cheese include pathogens reduction and extension of shelf-life.

More recent studies have indicated the feasibility of using irradiation in other traditional cheeses, especially for controlling *Listeria monocytogenes*.

7.1.1. Limitations to the use of food irradiation

Sensorial quality and other properties as nutritional ones could be affected after irradiation of different types of cheese and in consequence they should be considered as possible limitations of irradiation in this specific food (Ham et al., 2009; Konteles et al., 2009). For example, Konteles et al. (2009) reported that irradiation of Feta cheese at doses of 4.7 kGy resulted in alterations of its colour and aroma.

On the other side, and for other types of cheese, Tsiotsias et al. (2002) did not report any adverse sensory effect on the Anthotyros cheese after irradiation at 4kGy. Ju-Woon (2005) did not report any change in sensorial properties between frozen cheese balls non-irradiated and irradiated at doses up to 3 kGy, while some adverse sensory effects were described when irradiating with doses of 5 kGy or more.

7.2. Hazard identification

7.2.1. Microbiological agents to be considered

According to previously (Cecchi et al., 1996; SCF, 1992) and latest published works (Ju-Woon et al., 2005), the microorganisms of highest concern in this food class because of the potential risks they may represent to consumers are *Listeria monocytogenes*, *Escherichia coli*, *Salmonella* spp., *Clostridium* spp., *Staphylococcus* spp., fungi (because of mycotoxins), *Brucella* spp. and *Mycobacterium* spp.. *B. cereus* has also been identified by Lopez-Pedemonte et al. (2003) as pathogenic microorganisms that can be transmitted by some types of cheeses.

7.2.2. Comparison of the situation with the past

The use of raw milk in the production of cheese, faulty pasteurization or equipment and post-processing cross-contamination are still contributing factors of reported outbreaks in which cheeses are involved.

7.3. New efficacy data available in scientific literature

Some new studies have been carried out in relation to the kinetic of inactivation of different pathogenic microorganisms in soft cheeses.

Contaminated Feta cheese samples with *L. monocytogenes* were exposed to irradiation doses of 1.0, 2.5 and 4.7 kGy. None of the irradiation doses eliminated *L.monocytogenes* in the samples of cheese made with contaminated pasteurised milk and the reduction achieved was of 1.48, 2.36 and 3.8 $\log_{10}$

respectively. In experiments with post-process contamination (i.e. when 10^3 CFU/ml contaminated brine was used for packaging), irradiation at 2.5 kGy and 4.7 kGy reduced *L. monocytogenes* counts below the detection limit (Konteles et al., 2009). The D_{10} values for *L. monocytogenes* were 0.28 and 0.30 kGy when inoculated onto two types of sliced processed cheeses (Sommers and Boyd, 2005).

Tsiotsias et al. (2002) studied the feasibility of gamma irradiation at doses of 2-4 kGy for eliminating *Listeria monocytogenes* inoculated into the freshly produced soft whey cheese Anthotyros and the calculated D_{10} value for *L. monocytogenes* was 1.38 kGy.

Ju-Woon et al. (2005) investigated irradiation of fried-frozen cheese balls and concluded that irradiation at the dose of 3 kGy is an effective treatment to ensure microbiological safety of this product. The D_{10} value for *Escherichia coli* (KCTC 1682) was 0.25 kGy.

7.4. Irradiation and alternative preservation methods / technologies

7.4.1. Comparison with alternative preservation methods / technologies

High hydrostatic-pressure treatment offers the food industry an alternative technology for food preservation. Interest in high pressure application on milk has recently increased. Pressures between 300 and 600 MPa can inactivate microorganisms including most infectious food-borne pathogens. In fresh cheese, high hydrostatic-pressure treatment at 400 MPa was found to cause a reduction of 7 logs of *E. coli* populations (Trujillo et al., 2000).

The use of electrical pulses to pasteurize milk could be an alternative if heat is not a good solution (Sampedro et al., 2005).

8. Frog's legs

8.1. Objectives

In 1998, the Scientific Committee for Foods (SCF, 1986) recommended that frog's legs could be irradiated at doses up to 5 kGy. According to the SCF, at this dosage no nutritionally relevant radiolytic changes occur which are likely to cause a toxicological hazard, and there is no safety reason not to allow the decontamination of frozen frog's legs by irradiation up to an average maximum dose of 5 kGy.

8.2. Hazard identification

8.2.1. Microbiological agents to be considered

The most important hazard arises from contamination with *Salmonella* and other faecal pathogens occurring in frog's legs at the time of deep-freezing. *Escherichia coli* and *Staphylococcus aureus* have been also found in frog's legs. Although frog's legs are cooked for consumption there is a risk for cross-contamination (Andrew et al., 1977). Destruction of pathogenic flora of deep frozen product by irradiation at an average dose of 5.0 kGy has shown to be effective. A dose of 3.0 kGy is adequate to ensure hygienic quality from *Salmonella* and related bacteria in frozen frog's legs (Loaharanu, 1997).

8.3. New efficacy data available in scientific literature

Few new references related to irradiation of frog's legs are available. Santiago et al. (2005) evaluated the shelf-life of frog carcasses submitted to 3 kGy of γ-irradiation and kept at 4°C or -2 °C. Frogs

slaughtered under conditions based on the hygienic-sanitary criteria established by the Brazilian legislation were used. Irradiation of frog carcasses increased the storage life and decreased the levels of microorganisms, without modifying the sensorial quality and consumer acceptance of the product.

8.4. Irradiation and alternative preservation methods / technologies

8.4.1. Comparison with alternative preservation methods / technologies

Nickelson et. al (1975) used a chlorine ice bath (200 ppm) to decontaminate frog legs from *Salmonella* as part of a processing procedure and reported a significant reduction of *Salmonella* in the product.

9. Casein and caseinates

9.1. Objectives

In the 1990s, the use of irradiation for casein and caseinates was proposed as a means of decontamination of casein products such as acid casein, rennet casein and caseinates, to avoid any incidence of toxic infection of the consumer (SCF, 1998), since, despite good hygienic control, the final products may still contain *Cronobacter (Enterobacter) sakazakii*, *Escherichia coli*, *Salmonella* spp. and *Klebsiella pneumoniae*. This section will also consider infant formulas, which frequently contain dry dairy protein products and on which have been done many recent works with regard to irradiation. Besides inactivation of pathogenic microorganisms, a secondary objective of irradiation of this category of food can also be allergens destruction.

9.1.1. Limitations to the use of food irradiation

Probably the main limitation of irradiation of casein and caseinates is linked to its use in infant formulas because it affects their nutritional properties. Depending on the dose, irradiation can lead to the destruction of fat soluble vitamin A, vitamin B1 (Thiamine), vitamin C and essential amino acids. Nevertheless, heat could produce a higher destruction of those components when applied to achieve the same microbial inactivation level.

Lee et al. (2006) did not observe any marked sensory effect on dehydrated powdered infant formulas irradiated at 5 kGy after rehydratation and heating.

9.2. Hazard identification

9.2.1. Microbiological agents to be considered

The microorganisms of highest concern in this category of food are represented by S*almonella* spp., *Listeria monocytogenes*, *Escherichia coli*, *Cronobacter (*former *Enterobacter*) *sakazakii* and *Klebsiella pneumoniae*.

9.2.2. Comparison of the situation with the past

C. sakazakii has been associated with numerous cases of meningitis and necrotizing entercolitis in infants (EFSA, 2007b). This microorganism could be present in infant milk formulas. Additionally, even more caseinates are used to produce edible coating for foods.

9.3. New efficacy data available in scientific literature

Many of the more recent published works in relation to casein and caseinates are on the effect of irradiation of caseinate films used in coating regarding to their structure and microbiological contamination (Ciesla et al., 2006; Nortje et al., 2006). For instance, irradiation at 4 kGy reduced *S. aureus* by 6 $\log_{10}$ cycles in inoculated biltong (strips of dried meat) coated with edible casein-whey protein (Nortje et al., 2006). Nevertheless there are some new works on inactivation of microorganisms by using irradiation including infant formulas.

Lee et al. (2006) observed a decrease of approximately 3 $\log_{10}$ on *C. sakazakii* in the dehydrated powdered infant formulas by irradiation with 3.0 kGy or rehydration with hot water at 80 °C. No recoverable bacteria were found in the powdered infant formulas irradiated at 5.0 kGy and stored, either before or after rehydration.

Osaili et al. (2007) studied five strains of *C. sakazakii* that were inoculated individually into brain heart infusion broth and rehydrated or dehydrated infant milk formulas and exposed to ionising radiation. *C. sakazakii* strains were exposed to an irradiation dose of up to 1 kGy and 9 kGy for liquid and dehydrated samples respectively. D_{10} values (calculated by a linear regression model) ranged from 0.21 to 0.29 kGy, 0.24 to 0.37 kGy and 1.06 to 1.71 kGy in brain heart infusion broth, rehydrated and dehydrated formulas, respectively. The authors concluded that ionising radiation is able to inactivate *C. sakazakii* in rehydrated and dehydrated milk formulas. Another study reported a higher D_{10} value of 4.83 kGy (Hong et al., 2008). The authors suggest the much higher D_{10} value in their study could be due to differences in the formulation and this would need to be confirmed through further investigation.

Osaili et al. (2008) evaluated the effect of different environmental stresses on the resistance of *C. sakazakii* in powdered infant milk formulas towards the resistance to gamma radiation and concluded that most forms of environmental stress are unlikely to significantly enhance the resistance of *C. sakazakii* strains to irradiation treatment at doses up to 7.5 kGy. Results from an additional study showed that the length of the dry storage of powdered infant milk formulas increased the radiation resistance of *C. sakazakii*. The D_{10} value for *C. sakazakii* following overnight storage was 0.98 kGy but over 4 kGy were required to kill 1.5 $\log_{10}$ of the same strain that survived twelve months in dry powdered infant milk formulas, suggesting that irradiation should be applied shortly after manufacture.

9.4. Irradiation and alternative preservation methods / technologies

9.4.1. Comparison with alternative preservation methods / technologies

Studies carried out by Pina Pérez et al. (2007a) indicated that pulsed electric fields are effective against *C. sakazakii* in re-hydrated infant formulas, so it could be an alternative to the powder irradiation.

High Hydrostatic Pressure can be also used to pasteurize liquid infant formulas (Pina Pérez et al., 2007b).

10. Egg white and eggs in general

10.1. Objectives

In the 1990s, the use of irradiation for treatment of egg white, whether liquid, frozen or dehydrated by irradiation was asked in order to assure a bacteriological quality as needed by the food industry and to avoid secondary contamination of the decontaminated product during packaging, storage and transport

(SCF, 1998). The main objectives for irradiating these products include pathogen reduction and extension of shelf-life. Food irradiation is an alternative to heat pasteurization to reduce *Salmonella* spp. and *Campylobacter* spp. in eggs (Pinto et al., 2004). Another possible reason is the reduction of allergenicity of eggs and products containing eggs (Seo et al., 2004), improving of egg white foaming ability and quality of final bakery products (Song et al., 2009b).

In addition to the application to egg white, irradiation can also be applied more generally to eggs and egg products (such as egg yolk, egg blends, in-shell eggs).

10.1.1. Limitations on the use of food irradiation

The main limitations to the irradiation of eggs and egg products are in relation to the alteration of the nutritional and chemical characteristics of these products as well as aesthetical characteristics and difficulties in cooking (Al-Bachir and Zeinou, 2006; Hwang et al., 2001; Min et al., 2005).

10.2. Hazard identification

10.2.1. Microbiological agents to be considered

The microorganisms of highest concern in this category of food because of the potential risks they may represent for consumers are represented by *Salmonella* spp., *Campylobacter* spp. and *Staphylococcus* spp. (Alvarez et al., 2007a; Alvarez et al., 2006; Badr, 2006). In the case of liquid egg the pasteurization process should be enough to destroy these microorganisms, although it does not inactivate the *Bacillus cereus* spores. This is of concern for the production of cream-caramel or other similar derivates (Collado, 2008).

10.2.2. Comparison of the situation with the past

Liquid egg is widely used as raw material for many other processed products (e.g. bakery, ready-to-eat foods). Liquid eggs could be the source of salmonellosis cases if the product is not properly pasteurized and handled. Even more, *Bacillus cereus* is not inactivated by using the current thermal process and it poses a concern for end user companies.

10.3. New efficacy data available in scientific literature

Recent studies reported D_{10} values for *Salmonella* in shell eggs and liquid eggs between 0.5 and 0.65 kGy (Al Bachir and Zeinou, 2006; Alvarez et al. 2006), with some serovars (e.g. Senftenberg) being more resistant. Al-Bachir and Zeinou (2006) reported a D_{10} value of 0.45 kGy for *Salmonella* and concluded that eggs irradiated with 1.5 kGy may be suitable microbiologically to prepare safe mayonnaise. The indicated D_{10} values for *Salmonella* in shell and whole eggs are in the range of those reported in Chapter 3 of the main body of the present Opinion.

10.4. Irradiation and alternative preservation methods / technologies

10.4.1. Comparison with alternative preservation methods / technologies

Alternatively, liquid egg can be treated by pulsed electric fields to inactivate vegetative cells (Sampedro et al., 2006). Shell eggs could be refrigerated to control *Salmonella* and other pathogenic microorganisms.

10.4.2. Association with other preservation methods / technologies

Irradiation could be associated with other technologies in order to reduce the irradiation intensity, diminishing in this way its adverse effects on quality.

Alvarez et al. (2006) concluded that irradiation at doses lower to 1.5 kGy prior to thermal treatment would enable the reduction of heat treatment times by 86% and 30% at 55° and 57°C, respectively, and would inactivate 9 $\log_{10}$ units of *Salmonella* serovars.

Alvarez et al. (2007a) studied mathematical models describing the inactivation of *Salmonella* Enteritidis, *Salmonella* Typhimurium, and *Salmonella* Senftenberg suspended in liquid whole egg by irradiation followed by heat treatments and calculating D_{10} values following to these treatments at different doses and temperatures. The treatments obtaining a 5-$\log_{10}$ reduction on any of the investigated *Salmonella* serovars were determined to be 57.7-59.3ºC/3.5 min following 1.5-0.5 kGy depending on the medium where the treated cells were recovered. By comparison, current industrial liquid whole egg heat treatments (60 ºC/3.5 min) would inactivate 3 $\log_{10}$ cycles of the *Salmonella* population.

In a further study, Alvarez et al. (2007b) investigated the effect of combining irradiation and heat on *Salmonella* Enteritidis and *Salmonella* Senftenberg inoculated into liquid whole egg with a number of additives (added nisin, EDTA, sorbic acid, carvacrol, or combinations of them). Synergistic reductions of *Salmonella* populations were observed when liquid whole egg samples containing these additives were treated by gamma radiation (0.3 and 1.0 kGy), heat (57 and 60 ºC), or both treatments combined. The authors concluded that the synergistic reduction of *Salmonella* viability by irradiation and heat treatments in the presence of these additives could enable liquid whole egg producers to reduce the temperature or processing time of thermal treatments or to increase the level of *Salmonella* inactivation.

The effects of irradiation and cold storage on the microbial safety of liquid whole egg and liquid egg yolk was established during storage throughout the enumeration of the total plate count, enterobacteriaceae, *Staphylococcus aureus* as well as the detection of *Salmonella*. The results showed that gamma irradiation at 3 kGy was enough for improving the microbial safety of samples, and appeared to be the optimum for treating liquid whole egg and liquid egg yolk at room temperature followed by cold storage at 4 ºC (Badr, 2006).

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GLOSSARY

Dose or Absorbed dose (D)	Sometimes referred to simply as 'dose', is the amount of energy absorbed per unit mass of irradiated food product (CAC, 2003b).
D_{10} value or decimal reduction value or D-value	Dose of radiation required to reduce the viability of a population by 90% (1 $\log_{10}$ cycle) under the stated conditions.
Dose Limit	The minimum or maximum radiation dose absorbed by a food product prescribed in regulations as required for technological reasons. Such dose limits are 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).[23]
Dose uniformity ratio (U)	The ratio of maximum to minimum absorbed dose in the production lot (CAC, 2003b), irradiated as a single entity. $U = D_{max} \div D_{min}$.
Dosimetry	The measurement of the absorbed dose of radiation at a particular point in a given absorbing medium (CAC, 2003b).
Gray (Gy)	Unit of absorbed dose where 1 Gy is equivalent to the absorption of 1 joule per kilogram (FAO, 2008).
Half-value thickness	Layer thickness of a material reducing the intensity of radiation by absorption and scattering by half (European Nuclear Society[24]).
Maximum dose (D_{max})	The highest absorbed dose in a production lot irradiated as a single entity.
Minimum dose (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 Codex but not used in the current revised Codex General Standard for Irradiated Foods (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(D_{max}+ D_{min}). In general: $\overline{D} = \frac{1}{M} \int p(x,y,z) d(x,y,z)$ 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.

[23] 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".

[24] www.euronuclear.org/info/encyclopedia/h/half-value-thickness.htm (accessed on 21 September 2010)

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