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Revision of the opinion of the Scientific Committee on Food on the irradiation of food

(expressed on 4 April 2003)

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1. TERMS OF REFERENCE

To revise the opinion of the Scientific Committee on Food on the irradiation of food prepared in 1986 in the light of new developments.

In particular, to advise the Commission on the question

(1) whether it is appropriate to specify a maximum dose for the treatment of certain products

(2) whether it is appropriate to evaluate foodstuffs individually taking into account aspects like

- safety of irradiated foods for the health of consumers,
- technological need,
- no substitute for good hygiene and good manufacturing and agricultural practices,
- the need to specify conditions for high-dose irradiation.

2. BACKGROUND

The framework Directive 1999/2/EC concerning foods and food ingredients treated with ionising radiation lays down general and technical aspects for carrying out irradiation, the need to establish a positive list of products authorised for irradiation treatment and it includes the authorised maximum doses, the labelling of irradiated foods and the conditions for authorising food irradiation.

The treatment of a specific food item may only be authorised

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

In addition, the Directive lays down that foodstuffs may be only authorised for treatment with ionising radiation if the SCF has expressed a favourable opinion on this particular foodstuff and if the authorised maximum dose does not exceed the dose recommended by the SCF.

The list of foodstuffs authorised in the Community for treatment with ionising radiation appears in the implementing Directive 1999/3/EC on the establishment of a Community list of food and food ingredients authorised for treatment with ionising radiation. So far, this positive list contains only one single food category "dried aromatic herbs, spices and vegetable seasonings", which can be treated with a maximum overall average dose of 10 kGy. According to Directive 1999/2/EC, the Commission eventually has to submit a proposal for completion of the positive list to the European Parliament and the Council.

On the basis of scientific studies, the Food and Agriculture Organization, the International Atomic Energy Agency and the World Health Organization (FAO/IAEA/WHO) concluded in their 1981 report (FAO/IAEA/WHO, 1981), that any food irradiated up to a maximum dose of 10 kGy is considered to be safe and wholesome. As a consequence of this report a Codex General Standard for Irradiated Foods was adopted in 1983 and a Codex Recommended International Code of Practice for the Operation of Radiation Facilities used for the Treatment of Food was adopted in 1984.

Building upon the work of FAO/IAEA/WHO, the Committee expressed opinions on irradiated foods in 1986, 1992 and 1998 and gave favourable opinions on the irradiation of a number of foodstuffs (fruit, vegetables, cereals, starchy tubers, spices and condiments, fish, shellfish, fresh meats, poultry, camembert from raw milk, frogs legs, gum Arabic, casein/caseinates, egg white, cereal flakes, rice flour, blood products). The Committee emphasised that food irradiation must not be used to cover negligence in handling foodstuffs nor to mask their unsuitability for use as food.

A further development in this area of food technology has been the continued efforts of FAO, IAEA and WHO to update existing knowledge in this field. These resulted in a further review by WHO of the safety and nutritional adequacy of irradiated food, based on an appraisal of all relevant scientific studies carried out since 1980, at the request of one of the WHO Member States, in order to establish whether any of the controversial issues and claims of adverse nutritional effects of irradiated foodstuffs had been substantiated in the intervening period. A WHO report on this review was issued in 1994 (WHO, 1994). Its conclusions indicated that food irradiation was a thoroughly tested technology and that so far no deleterious safety aspects had been discovered. As long as good manufacturing practice was implemented, food irradiation was considered by WHO to be a safe and effective processing technology. The possible risks of deviations from good manufacturing practice in relation to irradiation were no different from those encountered by other food processing methods such as canning, pasteurisation and freezing.

Subsequently, a Joint FAO/IAEA/WHO Study Group on High-Dose Irradiation was convened in 1997 to

(1) review all relevant data related to the toxicological, microbiological, nutritional, radiation-chemical and physical aspects of foods irradiated with doses above 10 kGy, and to determine whether foods so treated are wholesome, and to

(2) consider whether a maximum irradiation dose needs to be specified.

This Study Group considered the wholesomeness and nutritional adequacy of foods irradiated with high doses, i.e. with doses above 10 kGy, the previously suggested limit, because of recent indications for the usefulness of irradiation to ensure the hygienic quality of food of animal origin, to overcome quarantine trade barriers in fresh fruits and vegetables, and the demand for high quality shelf-stable convenience foods for general use and for specific vulnerable population groups.

This Study Group concluded that the data on radiation chemistry, toxicology, microbiology and nutritional properties of foods treated with radiation doses greater than 10 kGy were adequate. It further concluded that food irradiated to any dose appropriate to achieve the intended technological objective was both safe to consume and nutritionally adequate. Since, in practice, the doses applied to achieve the intended technological conditions of good manufacturing practice would be such as not to compromise the organoleptic quality of the irradiated food, an upper dose limit was not required. The report of this study group was published by WHO in 1999 (WHO, 1999a).

3. RADIATION CHEMISTRY CONSIDERATIONS

3.1 Induced radioactivity

In general no fundamentally new knowledge has been published on this subject since the review of the 1986 SCF report. This report had concluded that no measurable radioactivity would be induced in irradiated food and that no health problems could be associated with this issue. The amount of radioactivity produced was found to be below the detection threshold and about 10^5 smaller than the amounts that are found naturally in fresh foods. Restricting the maximum permitted energy of electron generators to 10 MeV and of X-rays to 5 MeV ensured that no observable radioactivity could appear even with doses up to 50 kGy (Diehl, 1995). Neither ^{60}Co nor ^{137}Cs gamma rays, the commonest commercial radiation sources, reach energy values capable of inducing radioactivity. The calculated levels of induced radioactivity in a wide range of foods 24 hours after irradiation were below any level of concern (Terry and McColl, 1992).

3.2 Effects of irradiation

Food processing involves the input of thermal, mechanical, photonic or ionising radiation energy. There have been no fundamentally new developments in this area since the 1986 SCF report. It should still be noted, that the induced physical and chemical changes differed in their extent with the penetration of the energy applied and the amount absorbed but were relatively small and uniform (Taub, 1981; Basson, 1983; Diehl, 1995). Microorganisms are destroyed by OH· radicals formed in their cells reacting with their DNA, thereby breaking the chain bonds and blocking replication. Chronic toxic and genotoxic compounds can only form if pathways exist for their formation. The chances of degradation of micronutrients depend on the result of their competition with the other food constituents present for primary reactive radicals. The same situation holds for the relevant constituents responsible for organoleptic changes.

The yields and the nature of the resultant radiolytic products can be predicted from the known chemical composition of the irradiated foods. Even the largest radiation doses, however, result in very low levels of absorbed energy. The direct effects in the matrix following energy

deposition are high-energy processes occurring within 10^{-11} seconds, while indirect effects result from the reaction of the precursor radicals with minor matrix constituents to form stable radiolysis products.

Following localized interaction of radiation with constituent atoms of the treated foodstuff, the yields of radiolytic products generally increase linearly with radiation dose but also depend on the mass fraction of the affected component. The products distribute uniformly and react by homogenous kinetics (Farhataziz and Rogers, 1987). The nature of the radiolytic products formed is independent of the amount of radiation absorbed and ranges from excited to dissociated and ionised molecules. Most free radicals produced are short-lived or become almost instantaneously stable chemical entities unless formed in deep-frozen foods or dry solids, e.g. bones or shells, where the limited diffusion favours their persistence.

The irradiation of dilute aqueous solutions is more destructive than the direct effect of irradiation on dry solids. Irradiated multicomponent systems show less destruction of the individual components than irradiated pure solutions of single compounds. Both pH and temperature determine the radiolytic products formed. The product yields from reactions with primary precursor radicals are calculable. They are extremely small compared with heat-induced yields (WHO, 1999a).

3.3 Radiation effects on proteins

Further work has confirmed the previously reported findings relating to the radiation chemistry of proteins, peptides and isolated amino acids. Additional pathways for reactions with primary and secondary entities ($H^{\cdot}$, $OH^{\cdot}$, solvated electron) have been identified for metalloproteins, particularly myoglobin (Whitburn *et al*., 1982; Whitburn *et al*., 1984).

3.4 Radiation effects on lipids

This aspect is covered by the non-aqueous radiation chemistry of the cation radicals and excited molecules, that are produced initially by irradiation. Any palmitic acid present will also form 2-dodecylcyclobutanone at a level of 0.5 µg/g lipid at a radiation dose of 5 kGy (Boyd *et al*., 1991) and this compound can thus serve as a marker for irradiation treatment of lipid-containing foodstuffs. Other cyclobutanones may possibly be formed from the other fatty acids present.

3.5 Radiation effects on carbohydrates

No fundamentally new findings have been reported regarding the radiolysis products formed from carbohydrates, the formation of these radiolysis products being proportional to the dose applied (Raffi *et al*., 1985). The overall chemical consequences of the irradiation of carbohydrates in muscle food are minor because in these foods carbohydrates do not compete for primary radicals.

3.6 Radiation effects on vitamins

The losses following irradiation of pure vitamin solutions differ from those found in actual food and are generally greater in aqueous solutions. Some studies with vitamin C not previously reviewed have been reported (Nagay and May, 1985; Zegota, 1988; Bielski, 1982).

3.7 Radiation effects on inorganic salts

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

3.8 Radiation effects on nucleic acids

The reactions of primary radicals with the purine and pyrimidine bases in DNA cause damage and are important events for microbial destruction. Any altered bases are not competitors for normal bases during DNA synthesis because only base precursors are involved in DNA synthesis.

3.9 Chemiclearance

This concept was used in the 1981 FAO/IAEA/WHO evaluation of the safety of food. It facilitated the generic clearance of irradiated foodstuffs by basing it on the finding that foods of similar composition will respond similarly to irradiation. Thus wholesomeness established for one member of a class of irradiated foods could be extended to all similar members of the same class. Because the chemical responses to irradiation with doses >10 kGy of precooked, moist muscle foods have been found to be similar, these responses can be extrapolated to the generic class comprising these foodstuffs. Commonality of the nature and behaviour of intermediate radicals and of the yields of stable radiolytic products has been demonstrated by the similarity of the patterns of the ESR spectra of pork, ham, beef and chicken irradiated with doses greater than 10 kGy. The same observation holds for the triglycerides of meats irradiated with doses >10 kGy (Sevilla, 1994). These findings were considered useful in arguing for avoiding the performance of animal feeding tests on each individual irradiated food usually required for their safety evaluation.

Similar consistencies in radiation chemistry were shown for starches and glucose oligomers by the similarity of their ESR spectra and the dependence of their decay on water content and storage time (Raffi *et al*., 1987). Measurements of thiamine also showed consistency (Fox *et al*., 1995). These studies were done on samples frozen under elimination of oxygen in the ambient atmosphere, thus reducing any chemical processes to 20% of those occurring in a non-frozen aqueous phase. Because the residual oxygen in a muscle matrix is reduced by radiolysis during the absorption of the first 0.6 kGy fraction of any applied dose, all precooked, vacuum-packed foods irradiated with 50 kGy while frozen will show the same chemical changes as raw foods irradiated with 10 kGy either chilled or at ambient temperature in the presence of oxygen. Thus the end products of radiolysis may be predictable by commonality and extrapolatable from model compounds to food commodities irradiated at doses above 10 kGy. In the view of the WHO group this commonality also supported the assumption that irradiated food commodities other than meat might yield spectra of radiolysis products similar to those found at irradiation doses below 10 kGy. Any increase in dose would merely increase the level but not the spectra of radiolysis products. Therefore, other food ingredients of irradiated frozen meat products were unlikely to form novel chemical entities and did not need to be tested for wholesomeness (WHO, 1999a).

3.10 Total yield of radiolytic products

The total yield of radiolytic products depends largely on the magnitude of the absorbed radiation dose. Other factors are food temperature, viscosity, composition, and the gaseous

environment during irradiation. At a dose of 1 kGy, it has been calculated that the total yield of all radiolytic reaction products will amount to 30 mg/kg if the average relative molecular mass of the radiolytic products is 300 Da (WHO, 1994). This yield increases linearly with dose for the major food constituents but remains constant or decreases for minor constituents as these are depleted. Irradiating moist frozen foods in the absence of oxygen decreases the overall yields by 80% so that treatment at 50 kGy at -30°C is equivalent as regards yield to treatment with 10 kGy at chill temperature. Model systems lack the competitive reactions occurring in real foods. In the opinion of the WHO group practically all radiolysis products identified in foods irradiated at doses >10 kGy are either already naturally present or likely to be produced also by thermal processing (WHO, 1999b)

3.11 Unique radiolytic products

A re-examination of the list of 63 unique radiolytic products extracted from the literature by the FDA in their initial assessment of food irradiation showed that only 3 volatile hydrocarbons deriving from food lipids had not been subsequently found in non-irradiated foods. These had a chain length of 1C atom less than their homologues in untreated foods. In the view of the WHO group it was likely that most non-volatile radiolytic products will eventually also be found to be constituents of non-irradiated foods. Any unique radiolytic products derived from the coupling of lipid and protein radicals are likely to be hydrolysed enzymatically during digestion. Heating fatty acid esters produces the same dimers as does irradiation. In these circumstances it was the view of the WHO group that the concern over unique radiolytic products was probably unwarranted and extensive searches unrealistic in view of the vast number of different types of food (WHO, 1994). Further work has produced evidence that alkylcyclobutanones could be specifically detected in low yields in irradiated foods as markers of this processing technique. These cyclic compounds could in the opinion of the WHO group also be both produced and decomposed in lipids subjected to high temperatures (WHO, 1999a). See also section 6.4 for further details on the toxicological properties of these compounds.

4. NUTRITIONAL CONSIDERATIONS

Recent investigations have confirmed the commonality and predictability of radiation effects. Any new aspects reported are concerned with foodstuffs irradiated at doses of 10-45 kGy, on which much early work was done by the US Army investigation into producing readily available provisions containing meat, poultry, fish and shellfish of good nutritional quality capable of being stored at ambient temperature for prolonged periods. This technology had already been used to provide foods suitable for patients on immunosuppressive therapy and was reconsidered for general use by the US Army in 1992 and for use by some special consumer groups requiring this type of convenience food.

Doses of irradiation much above 10 kGy degrade a variety of nutrients similar to the application of thermal energy, so that ultimately the overall effects are similar (Diehl, 1991; Diehl *et al*., 1991). Most research indicates that vitamin losses can be minimised by irradiation in oxygen-free packaging (cans or flexible pouches) or by irradiating at cryogenic temperatures ranging from -20°C to -40°C. However, the enormous variations likely to occur in cooking practice prevent any accurate prediction of total nutrient loss. Solutions of vitamins in water are more vulnerable to destruction by irradiation than vitamins in the food matrix or in dehydrated foods. Hence extrapolation of nutrient loss from aqueous solutions to

solid food is inappropriate. Vitamins in dry spices are very resistant to destruction by irradiation (Murray, 1983).

4.1 Macronutrients

Using protein efficiency ratio (PER) determination of irradiated meat by rat growth assays, no adverse nutritional effects of chicken meat, irradiated at 59 kGy, (Thayer *et al*., 1987) nor any changes in amino acid pattern were observed (Thayer, 1990). Corn protein and wheat gluten irradiated at 28 kGy showed no deleterious effect on their biological value but in chick growth assays the nutritional value was improved. Similarly, absence of adverse nutritional effects were noted with rat diets irradiated up to 70 kGy and with legume seeds irradiated up to 210 kGy.

4.2 Water-soluble vitamins

The most radiation-sensitive vitamin of this group is thiamine, the least radiation-sensitive is nicotinic acid. Additional data concern the loss of tetrahydrofolic acid in pulses irradiated at 25 kGy with no effect on folic acid, dihydrofolic acid, 5-methyltetrahydrofolic acid and 4-formyltetrahydrofolic acid (Muller, 1991). Cobalamin is relatively resistant to radiation as noted in haddock fillets treated with 25 kGy, in other fish treated with 30 kGy, in pork chops treated with 6.65 kGy and in dairy products treated with 40 kGy at -78°C in a nitrogen atmosphere (Diehl *et al*., 1991; Thayer, 1990).

Additional results have been reported by Murray (1983). Irradiation with doses ranging from 30 kGy to 75 kGy, even when packaging under nitrogen and freezing at -78°C was used as ancillary measures, mainly affected thiamine and not riboflavin, niacin and folic acid particularly in ground beef, chicken and bacon. This was also noted in dairy products like mozzarella, cheddar, yoghurt and non-fat dried milk (Thomas *et al*., 1986; Dong *et al*., 1989; Thayer *et al.,* 1989; Thayer, 1990; Pfeiffer *et al.,* 1994; Muller and Diehl, 1996). It should be noted that vegetables, which are the main folic acid source, are not usually irradiated.

Since vitamin C activity includes also that of dehydroascorbic acid, the change of ascorbic acid to dehydroascorbic acid by irradiation is nutritionally of no significance. The vitamin C dietary sources in fresh fruits, fruit juices, vegetables and potatoes are unsuitable for treatment with irradiation doses above 10 kGy, because of the induced sensory changes, so no nutritional problems are likely to arise. It had been previously reported that onion powder, irradiated at 20 kGy, or ground paprika, irradiated with doses >10 kGy did not lose any vitamin C. (See also section 3.6).

4.3 Fat-soluble vitamins

The radiation sensitivity of these vitamins is variable, vitamin E being the most sensitive and vitamin D the least sensitive, overall effects of irradiation depending also on post-irradiation storage conditions (Daghir *et al*., 1983). Radiation-sensitive vitamins can be protected by using the following procedure: enzyme inactivation through heating to 73°C-80°C, vacuum packaging and irradiating at -25°C. The main dietary sources for vitamin E are butter, margarine, plant fats and oils, but as these foodstuffs are not suitable for irradiation because of the induced organoleptic changes, irradiation processing of foods makes no impact on the nutritional status of the consumer.

Vitamin A is also sensitive to destruction, if irradiated in air at ambient temperature. Losses can be minimised by vacuum packaging or the use of a nitrogen atmosphere and cryogenic temperatures during irradiation. Most food sources of vitamin A and carotenes such as milk, butter and cheese are unsuitable for treatment by irradiation at doses above 10 kGy. No evidence has ever been found for the production of antimetabolites by irradiation (Diehl, 1995; Skala *et al*., 1987).

Vitamin D is less sensitive to irradiation than vitamin A. No loss of vitamin D has been observed in chicken meat irradiated at -25°C with 59 kGy (WHO, 1999a).

Vitamin K is reasonably radiation-resistant in broccoli, cabbage and spinach irradiated at 28 kGy and 56 kGy and stored up to 15 months at room temperature. However, because of the low levels occurring in beef, irradiation at 28 kGy and 56 kGy can cause vitamin K deficiency if irradiated beef is the main source for its supply in the diet. When such irradiated beef is fed to rats as main component of their diet, a haemorrhagic syndrome occurs. Even in chicken meat irradiated at 59 kGy at -25°C some 36% of vitamin K is lost (WHO, 1999a).

4.4 Other micronutrients

Polyunsaturated fatty acids (PUFAs) were only lost to a small degree in cereal grains (rye, wheat, rice) irradiated at 63 kGy in air (Vaca and Harms-Ringdahl, 1986) and in herring fillets irradiated at 50 kGy (Adam *et al.*, 1982). Linoleic acid in soya beans was reduced by 16% following irradiation with doses over 100 kGy (Hafez *et al*., 1985). No effects on PUFAs were noted in chicken meat irradiated with 59 kGy at -25°C (Thayer, 1990). Hence high-dose irradiation had only marginal effects on these essential fatty acids.

5. MICROBIOLOGICAL CONSIDERATIONS

Treatment of foodstuffs with ionising radiation always has microbiological implications because of the effect of radiation on any microorganisms present in the food. One of the major objectives is to improve the safety of raw or minimally processed food by eliminating pathogenic microorganisms in the food. Initially the technological interest centred on control of insect infestation and the ripening and sprouting of fruits and vegetables. A further development was the use of irradiation to produce shelf-stable (commercially sterile) food products. Factors like type and number of organisms and intrinsic and extrinsic conditions affect the critical limits of radiation doses needed to achieve these objectives.

Irradiation doses above 10 kGy are used for shelf-stable high moisture foods of animal origin, for complete meals for persons on immunosuppressive therapy, astronauts, army personnel, individuals on special outdoor activities, and for the decontamination of low-moisture spices, herbs and dried vegetables (Farkas, 1988). This sterilisation technology is usually combined with vacuum packaging, mild heat treatment (73°C to 77°C) to inactivate proteolytic enzymes, and deep freezing during irradiation. The dose chosen should be capable of reducing the number of *Clostridium botulinum* spores by a factor of 10^{12} (12D).

When irradiation is used for pathogen control in HACCP-based systems, the critical limits for the dose applied for microbial destruction have to be calculated in relation to the microorganisms of concern and to the intrinsic and extrinsic conditions in the food product. Irradiation doses required simply for a reduction of viable pathogens in food products are lower than those needed for producing shelf-stable products. Doses ranging from 0.27 kGy at

5°C to 0.42 kGy at -5°C produced a 90% reduction (one $\log_{10}$ or D_{10}) in viability of VTEC O157:H7 in mechanically deboned chicken meat. Since this organism is found in low numbers, it was concluded that an irradiation dose of 1.5 kGy would achieve a 4-5 $\log_{10}$ reduction of VTEC and in practice eliminate it from meat (Thayer and Boyd, 1993).

5.1 Inhibitory effects of irradiation

The critical radiation target is the chromosomal DNA, damage to which inactivates the microorganism. About 90% of the damage is caused by OH· radicals released from the hydration layer around the DNA molecule. Usually the purine and pyrimidine bases are chemically changed and the phosphodiester backbone is broken (single-strand breaks) but some 5-10% double-strand breaks also occur (Moseley, 1989). Radiation-resistant organisms can also withstand effects on the plasma membrane (Grecz *et al*., 1983). The rate of dose absorption has no effect on survival except when oxygen replenishment is involved. Elevated temperatures above 45°C are synergistically bactericidal because the higher temperatures damage the repair systems. To achieve this effect in spores, temperatures of 80°C-98°C are needed. Subfreezing temperatures raise the radiation resistance of vegetative cells as the water activity decreases and the diffusion of radicals is restricted.

So far, there has been no evidence for the occurrence of mutations leading to more virulent, radiation resistant, strains; on the contrary, irradiation has been found to cause loss of virulence and infectivity as mutants are usually less competitive and less adapted (Farkas, 1992). Mutations can also be caused by thermal processing, preservatives and drying (Moseley, 1992). Single irradiation treatment hardly induces mutations to radiation-resistant survivors. The isolation of radiation-resistant mutants from wild-types is almost impossible because of the adequacy of the DNA repair capacity of wild strains. To develop radiation-resistant populations requires multiple cycles of irradiation. This was successful only with *Salmonella enterica var.* Typhimurium LT2, where two genes were mutated following this selection procedure (Ibe, 1982). Many cycles of heat treatment can achieve the same objective but no problems have appeared so far in pasteurisation plants. As most characteristics of microorganisms remain unchanged after irradiation, identification is not impaired (Finegold and Martin, 1982). Also, diagnostic and therapeutic irradiation has thrown up no diagnostic problem (WHO, 1994).

5.2 Critical limits

The inactivation response to irradiation follows the kinetics of the response to heat. Inactivation of the microorganisms is expressed as the reduction of the initial count in a power of 10^x (D_{10}). A large number of D_{10} values have been determined and published (WHO, 1999a). The most sensitive bacteria needing the lowest radiation doses for elimination are *Yersinia* spp., *Pseudomonas* spp., *Campylobacter* spp., *Aeromonas* spp., vegetative forms of *Bacillus cereus*, *E. coli* including O 157:H7 and *Arcobacter butzleri* (D_{10}~0.20 kGy) (Molins *et al.,* 2001). Relatively resistant to irradiation are *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, vegetative forms of *Clostridium perfringens*, *Enterococcus faecalis* and *Moraxella phenylpyruvia* (D_{10}~0-40-0.80 kGy) (WHO, 1999a; Molins *et al.,* 2001). The most resistant organisms are those in the non-sporeforming *Moraxella-Acinetobacter* group. They occur in the normal flora of meat and only cause spoilage in marine fish and shellfish. Others have been known for years, e.g. *Deinococcus radiodurans*, formerly known as *Micrococcus radiodurans,* which behaves like a Gram-negative bacterium. They possess efficient DNA repair mechanisms. They are not pathogenic and are sensitive to heat (Grant and Patterson, 1989; Oyaizu, 1987).

Some surviving *Clostridium* and *Bacillus* spores are resistant to irradiation, heat and chemicals and therefore may present a health hazard in irradiated foods. *Clostridium botulinum* type A and B spores are most resistant (D_{10} up to 2.79 kGy). Heat and radiation resistance do not correspond (Kim *et al*., 1987). The combination of irradiation with heat and additives is more effective because irradiation sensitises spores to heat inactivation especially at radiation doses above 10 kGy. The minimum required dose (MRD) must achieve a theoretical reduction of viability of 12 $logs_{10}$ (12D).

Radiation effects on specific parasites have been investigated (King and Josephson, 1983; CAST, 1989; IAEA, 1991; Loaharanu and Murrell, 1994; Wilkinson and Gould, 1996). Only doses <10 kGy are needed for inactivation of fish-borne, snail-borne and crustacean-borne parasites, liver flukes, *Paragonimus* spp*., Angistrongylus* spp*., Heterophyes* spp., *Anisakis* spp*., Trichina* spp*., Entamoeba histolytica* infection, *Hymenolepis nana.* and *Ascaris lumbricoides* eggs. Therefore sequential heating and freezing plus irradiation at doses above 10 kGy will inactivate all parasites (WHO, 1999a).

Since many yeasts have low resistance to irradiation, most yeasts are inactivated by doses up to 5 kGy but lower doses are effective at raised temperatures. Since the survival plots often have extensive shoulders higher doses may be necessary for D_{10} reductions. Since yeasts are non-pathogenic, the outgrowth of survivors carries with it no biological hazard except for causing spoilage (WHO, 1999a).

In the case of moulds and their mass of hyphae it is difficult to use the concept of reduction in numbers unless it is applied to conidia. Some D_{10} values for various mould spores have been determined. Moulds show differences in sensitivity to electron beam versus gamma ray irradiation, the latter being more effective in reducing spore numbers. Irradiation in the dry state was less effective and needed higher doses to reduce spore numbers. Irradiation of high moisture foods with doses >10 kGy appears to remove most moulds but some moulds in dry foods can survive doses above 10 kGy (Sharma *et al*., 1990; Blank and Corrigan, 1995; Chelack *et al*., 1991; van der Riet and van der Walt, 1985; Kumee *et al*., 1983; Szekely *et al*., 1991; Paster *et al*., 1985; Saleh *et al.*, 1988).

Viruses are more radiation resistant than bacteria with D_{10} values up to 4-5 kGy, however with a variability due to several factors. Small human viruses (hepatitis A) were found to be as radiation resistant as foot and mouth disease virus (WHO, 1995). Only after certain changes can a radiation-damaged virus be harmful. The heat pretreatment associated with irradiation of high moisture foods with doses >10 kGy is likely to inactivate food-borne viruses (WHO, 1999a). Some viruses would survive in irradiated dry commodities but their total number would probably be reduced (WHO, 1999a)

5.3 Intrinsic and extrinsic influences

In general microbial sensitivity to irradiation is greater at ambient temperatures than at freezing temperatures. The frozen state doubles the radiation resistance of vegetative bacteria. For *Pseudomonas* and *Acetobacter* the increase in resistance is 6.7 fold and rises to 8.8 fold if freezing is combined with anoxia. The radiation resistance of spores is little affected by freezing because the coat cortex and the forespore stage form a protective barrier against extracellular radicals. Complex media show component competition for the radicals produced by irradiation and thus provide protection for the microorganisms. A D_{10} reduction in VTEC

O157:H7 in ground beef requires 0.21 kGy and 0.31 kGy at temperatures of 4°C and -16°C respectively (Clavero *et al.,* 1994).

Over the pH range 5-8 the bacterial spore resistance to irradiation is unaffected but below pH 5 the sensitivity is increased. Chemical preservatives, e.g. curing salts, also sensitise bacterial spores to irradiation because they attract solvated electrons. Generally, the survivors of irradiation are more sensitive to temperature, pH, nutrient deficiencies, etc. than untreated bacterial cells, this being the rationale for combination treatments allowing a reduction in the required doses.

Non-spore forming pathogens and *Vibrio* spp are radiation-sensitive in frozen foods and do not survive irradiation doses above 10 kGy. Radiation tolerance is greater in aqueous model systems than in goods frozen and vacuum packed in an oxygen-free atmosphere.

5.4 Microorganisms/Commodity concerns

The cellular damage from irradiation of fruits and vegetables could lead to more rapid spoilage and if this was a major problem then these commodities could not be irradiated (Murray, 1990). The concern over the misuse of irradiation to sanitise unacceptably contaminated spoiled food has no real basis, as irradiation does not restore the appearance and the organoleptic characteristics of the spoiled food. In the opinion of WHO the reduction of spoilage and of pathogenic organisms extends shelf-life and reduces food-borne illness (Käferstein, 1990). Concern had been expressed in the early days of food irradiation, that the preferential destruction of normal spoilage organisms would permit unchecked growth of pathogens in food which would despite this still appear to be fit for consumption. Hence studies were requested to establish the safe use of irradiated food after treatment, in commerce and at home (ACNFP, 1986). Early fears were also expressed, that irradiation eliminating *Salmonella* spp., *Yersinia* spp. and *Campylobacter* spp. may allow *Clostridium botulinum* spores to survive (FDA, 1991).

In a study on chicken irradiated with 3 kGy the skins were inoculated with *Clostridium botulinum* type A and B. No toxin was found in chickens or controls kept at 10°C but toxin was present in those kept at 30°C. Here, toxin production was delayed in the irradiated chicken until after off-odours appeared indicating spoilage (Dezfulian and Bartlett, 1987). Since both the vegetative organisms and toxins are heat-sensitive, cooked fish presents no hazard but smoked raw fish may be a potential health risk.

It was found that in ice cream at -72°C, VTEC O157:H19 showed a D_{10} reduction at 0.2 kGy and that a low irradiation dose (1 kGy) could eliminate the natural number of pathogens present in ice cream without affecting the organoleptic quality (Kamat *et al.,* 2000).

5.5 Effects on microbial toxins

Increased ochratoxin production has been reported after irradiation of *Aspergillus ochraceus* (Paster *et al*., 1985). Usually decreased aflatoxin production has been found. Variable results are due to the heterokaryotic nature of *Aspergillus* spp. Inoculum size also affects toxin formation and this parameter had not been considered in earlier studies (Odamtten *et al*., 1987).

The available data indicate no risk of increased aflatoxin production (WHO, 1994). If mycotoxin levels are reduced they are affected to a lesser extent than the other constituents

and therefore cannot compete for primary radicals. Good manufacturing practice and good storage practice are still necessary after irradiation treatment.

Bacterial toxins are rather radiation-resistant in the food matrix and also when suspended in bacteriological media. Relevant data relate to *Clostridium botulinum* type A neurotoxin and to staphylococcal enterotoxin A (Rose *et al*., 1988; Modi *et al*., 1990). Preformed mycotoxins present in food are resistant to irradiation but can be removed by very high doses (Temcharoen and Thilly, 1982). The combination with H_2O_2 treatment was shown to be more effective in removing mycotoxins (Patel *et al*., 1989). Pure ochratoxin dissolved in methyl alcohol is stable to irradiation with 75 kGy (Paster *et al*., 1985).

5.6 Predictive modelling

Predictive modelling may help with estimating the destruction of spores by irradiation because it enables the avoiding of the time-consuming and costly inoculated pack studies. In the case of irradiation with doses >10 kGy knowledge of the variation of the chemical and physical factors in the food should be included in the modelling. More information on this procedure is still needed (Baranyi and Roberts, 1995; Farkas, 1994; McMeekin *et al*., 1993; Int. Comm., 1996; Whiting and Buchanan, 1997; Thayer and Boyd, 1993).

6. TOXICOLOGICAL CONSIDERATIONS

6.1 Subchronic studies

Over 400 feeding studies were available up to 1982 and were reviewed by the FDA for their acceptability as evidence for safety submitted to the WHO expert group of 1994. Of these, some 250 were considered acceptable or acceptable with reservations because of the latter's partial inadequacy. The deficiencies noted by the FDA concerned nutritional problems related to the animal diet used and inadequacies of the experimental design. Some 34 FDA-reviewed subchronic feeding studies were considered acceptable or acceptable with reservations. Of these feeding studies 26 were carried out in rats, 2 in mice and 6 in dogs. Only few adverse effects were reported, mostly in studies using radiation doses >10 kGy, and these were associated with the destruction of vitamins or other micronutrients in the laboratory animal diet. The adverse effects in the dog studies were seen only with feeds treated with irradiation doses <10 kGy and could not be evaluated because of the small number of animals used per group. Lack of dose-response relationships and marginal statistical significance made interpretation difficult in these studies. In a 90-day subchronic study, F_{2b} litter rats from a multigeneration study were fed either irradiated (3 or 6 kGy) or unirradiated chicken meat at 35% of the basal diet. No significant adverse effects were reported on body weight, organ weights, haematological and urinary parameters, nor any adverse gross or histopathological changes (FDA, 1987).

6.2 Reproduction and developmental toxicity studies

Of the 22 reproduction and teratology studies reviewed by the FDA some 11 were carried out in rats, 6 in mice, 3 in dogs and one each in hamsters and rabbits. A Dutch study found no difference regarding growth, reproduction, haematology and histopathology between rats fed either an irradiated laboratory diet (50 kGy) or an autoclaved laboratory diet (Strik, 1986). A subsequent three generation study in pigs using the same laboratory feed found no treatment-related effects in growth and reproduction parameters (Strik, 1986). Another multigeneration

study in rats compared irradiated (3 or 6 kGy) with unirradiated chicken meat at 35% in their basal diet. No treatment-related effects on reproductive parameters (fertility, pup number per litter, post-implantation losses) were observed and no adverse effects were reported on pup weight, pup mortality and pup growth rate (FDA, 1987)

6.3 Chronic/Carcinogenicity studies

Some 63 relevant studies were reviewed by the FDA and classified by the FDA as chronic studies. Of these, 32 were performed in rats (365-999 days), 18 in mice (365-800 days), 11 in dogs (365-999 days), and one each in the pig (300 days) and monkeys (730 days). Of the rat studies, 26 were carried out over periods sufficient to allow proper assessment of carcinogenicity. Many of them involved high irradiation doses, e.g. 25, 55.8, 56, 59, 60, 74 and 93 kGy. Irradiated individual foods, mixtures of foods and whole diets were fed. According to the FDA no treatment-related increase in tumours was seen in any of the studies and no significant toxicological findings were noted in the rat studies except the occasional reduction in a serum enzyme level or small decreases in pup weight in the second and third generation which corrected themselves after weaning. No consistent adverse effects were noted by the FDA in the mouse studies, the auricular dilation observed in one study could not be confirmed by a subsequent extensive histological survey on the hearts of some 5000 mice of the same strain fed irradiated diets.

According to the FDA most of the chronic studies in dogs showed either no or inconsistent adverse effects. The reported incidence of thyroiditis in one study using irradiated wheat could not be ascribed with any certainty to the treatment since thyroiditis is relatively common in beagles. No adverse effects were reported in the chronic pig and monkey studies.

In a further study the F_{1a} generation of pigs used in the reproduction study reported above was processed into ham products with or without nitrite, irradiated at 37 or 74 kGy and then fed to rats. Again no treatment-related effects of any kind were noted and no change in nitrosamine concentration due to irradiation was found (Strik, 1986). In earlier long-term feeding studies in rats, using composite food diets irradiated at 55.8 kGy, the only effect produced was an increase in cytochrome oxidase activity when no fruits or vegetables were present in the diet. No treatment-related effects on reproductive performance were noted, any variation probably having been due to nutritional factors rather than irradiation (USFDA, 1986). In a two-year study in rats, fed a diet containing either irradiated (3 kGy or 6 kGy) or unirradiated chicken meat at 35% in the diet no consistent or distinct differences between controls and test animals were observed in the usual parameters examined in a long-term study. No evidence of gross or histopathological abnormalities related to treatment was noted. The use of a small level of ethoxyquin on post-irradiation meat as antioxidant was considered not to be a confounding factor by the FDA (FDA, 1987).

6.4 Genotoxicity studies

Almost 60 studies on the induction of mutagenesis by irradiated foods were reviewed by the FDA. About 20 were designed to assess the potential to induce dominant lethal mutations in rats and mice. Only a few reported positive results after feeding wheat irradiated at 0.75 kGy while other studies, using much higher doses, gave negative results. Many studies were concerned with investigating the production of polyploidy following the reported observation that malnourished Indian children consuming wheat irradiated at 0.75 kGy for 4-6 weeks showed polyploid cells in their blood. A series of further studies failed to provide convincing evidence that feeding wheat irradiated at 0.75 kGy produces karyotypic abnormalities in the

bone marrow or lymphocytes in mammalian species. In addition, no increased incidence of polyploidy was reported in bone marrow cells and reticulocytes of Chinese hamsters fed wheat irradiated at 15 and 30 kGy (Tanaka *et al.*, 1992).

Irradiation of pure solutions of glucose or sucrose have been shown to produce mutagenic effects in the *Salmonella enterica var.* Typhimurium reversion test, to cause chromosomal aberrations in human lymphocytes and to induce mutations in *Drosophila melanogaster.* No such positive effects, however, were seen in any of the *in vivo* studies with irradiated foods. Induction of chromosomal aberrations in bone marrow or spermatogonia of mice could not be demonstrated.

The possible mutagenic activity of 2-alkylcyclobutanones, formed in fat containing foods during irradiation was recently considered by the Committee (SCF, 2002). These compounds are known to arise from irradiation-induced scission of the triglycerides in the food fat. They had been used as specific markers of irradiated fatty foods as early as 1992 but only recently has sufficient pure material been synthesised for carrying out tests for their genotoxic potential. Using reverse mutation tests in standard strains of *Salmonella enterica var.* Typhimurium, comet assays in cultured human colon cancer cells for DNA single-strand breaks, assays for cytotoxicity in human colon cancer cells and an *in vivo* assay for promoter activity in rat colon mucosa cells appeared to suggest an inherent genotoxic potential to be present in 2-alkylcyclobutanones (Burnouf *et al*., 2002). In some cases, however, the *in vitro* studies required cytotoxic concentrations to produce some effects. The 2-alkylcyclobutanones were found not to be genotoxic in the standard bacterial reverse mutation assay but were not tested *in vitro* for gene mutation or the induction of chromosomal aberrations in standard cultured mammalian cell assays nor were any animal feeding tests carried out. Therefore, the genotoxicity of these compounds could not be considered as having been established. The cytotoxicity was noted at concentrations of 0.30-1.25 mg/mL medium but these concentrations were about three orders of magnitude greater than the levels of 17 µg/g reported in the lipids of chicken meat irradiated at 59 kGy (WHO, 1999a). In contrast, no mutagenic activity was detected in earlier studies with *Drosophila melanogaster* and mice fed chicken irradiated at 55.8 and 59 kGy (Lusskin, 1979; Raltech Scientific Services, 1978).

6.5 Human clinical studies

In an early series of carefully controlled studies carried out in young male volunteers of the US Army, only briefly mentioned but not evaluated in detail in the 1986 SCF report, some 54 different high-dose irradiated foods (25-40 kGy, canned or frozen during irradiation and subsequently thawed and stored at room temperature) were administered for 15 days, followed by a control and washout period. Unirradiated control foods, otherwise similarly processed, were then administered to the same volunteers acting as their own controls. The experimental design provided for 35%, 60%, 80% and 100% of the metabolisable energy to be replaced by the irradiated foods. No toxic effects were noted irrespective of the proportion of irradiated food in the diet and no clinical changes were observed up to one year post-exposure (Bierman *et al*., 1958).

In a second early series of studies in volunteers a diet containing 32% of the calories replaced by either unirradiated or irradiated canned pork (30 kGy, canned and stored 1 year at room temperature) was administered for 15 days. The irradiated diet was not supplemented with vitamin K. No adverse clinical effects or prothrombin time abnormalities were noted (Plough *et al*., 1957).

In a third series of experiments volunteers consumed a variety of foods irradiated at 25-40 kGy and stored for three months at room temperature. Three daily menus supplied about 80% of the calories from the irradiated foods. Potatoes, flour and oranges were treated with 0.1-1.5 kGy while the other dietary components were treated at 25-40 kGy. No clinical abnormalities were detected (Bierman *et al*., 1958).

In all these studies the control foods could be distinctly distinguished by participants from the treated foods because of the changes in flavour, odour or texture of the test foods (Bierman *et al*., 1958). Overall no adverse clinical findings or in the clinical chemistry values were noted. Particular attention was paid to cardiac performance, haematology, hepatic and renal function.

In a well designed and carefully controlled, double-blind clinical study, involving 36 male and 34 female healthy students, some 35 different kinds of irradiated food were administered for 90 days. The same unirradiated diet was fed to a control group. The foods tested included two kinds of grain, 10 kinds of beans and bean products, about 20 kinds of fruits and vegetables, about 30 kinds of meats, fish, eggs and poultry, and 10 kinds of flavourings. The irradiation doses for meats were 8 kGy, for bean products, dried dates, lotus seeds and day lily 1-1.5 kGy, for rice, flour, soybean, red bean, peanut, mushrooms, other fruits and vegetables less than 1 kGy. The storage periods for the irradiated diets were not listed. The average daily diet contained 40 g meat, 300 g fruits and vegetables, 470 g grains, with some 60.3% of the total diet consisting of irradiated foods. Caloric and nutrient intakes complied with recommended values. Physical examination was carried out before and after diet consumption. In addition, numerical and structural chromosomal aberrations, sister chromatid exchanges (SCEs) and micronuclei in lymphocytes were determined. The urine was tested for induction of reverse mutations in *Salmonella enterica var.* Typhimurium strains TA98 and TA100 with and without metabolic activation. No adverse effects were noted in the physical examination. Non-significant differences in the frequency of chromosomal aberrations were seen in the test and control groups and there was no significant increase in polyploidy in the test groups compared with controls. Although the polyploidy incidence was higher after completion of the study in both test and control groups, it was not treatment-related. There were no significant findings either for the micronuclei or the SCEs. The urine showed no evidence of any mutagenic activity (Anon, 1987; Shao and Feng, 1988).

No other additional human clinical studies with irradiated foodstuffs have been reported.

7. COMMENTS

Review of the nutritional aspects has shown, that each irradiated food requires an individual evaluation of the radiation dose, the processing and storage and generalisations on this aspect are not possible. The nutritional significance of vitamin losses by irradiation depends on the proportion of the irradiated food in the total diet. Concerns would only arise in the unlikely situation where most of the total diet would be provided by irradiated food (WHO, 1994).

Irradiation with doses above 10 kGy is unlikely to present special microbiological problems as selective destruction of microorganisms or the induction of mutations are irrelevant in this process. Use of this process for precooked and pre-packaged high moisture food renders it shelf-stable and microbiologically safe. Spore survivors in high-dose (up to 30 kGy) decontaminated dry foods do not grow at low water activity and, because of the damage sustained, are more sensitive to heat, salt and pH. Good manufacturing practice is needed for safe end products. Radiation-sensitive vegetative forms of pathogens are subject to 12D

inactivation procedures. About 45 kGy for uncured meats and 30 kGy for cured meats produce a 12D reduction in radiation-resistant spore formers of *Clostridium botulinum*. Viruses are radiation-tolerant but heat-sensitive and are thus inactivated by the combination of the heat treatments used with foods irradiated at doses above 10 kGy.

Post-irradiation detection methods have been developed and standardised for a wide range of foods which may be subject to irradiation treatment (European Committee for Standardisation, CEN). Of particular importance in this connection are ESR spectroscopy and thermoluminescence as well as photoluminescence methods, and the detection of specific radiation-induced volatile hydrocarbons and 2-alkylcyclobutanones. In addition, some screening methods have been standardised.

8. CONCLUSIONS

As the toxicological and nutritional database relating to foods irradiated below 10 kGy has not been enlarged to any significant degree since the appearance of the 1980 FAO/IAEA/WHO and the 1986 SCF Reports, the Committee considered it not possible, at present, to deviate from its earlier position, that only those specific irradiation doses and food classes should be endorsed, for which adequate toxicological, nutritional, microbiological and technical data are available. The human clinical studies with irradiated foods, although they did not show any adverse effects following the consumption of irradiated foods, do not provide a sufficiently wide database to support a general extension of irradiation with doses up to a maximum overall average dose of 10 kGy to any foodstuff as being safe and wholesome. These studies were so far confined to foodstuffs consumed as part of either an European or Western diet and did not include any exotic or unusual dietary items consumed elsewhere nor did they include novel convenience foods, in which unusual or novel ingredients might be used as components. Since neither adequate compositional data covering any unusual dietary components used in the production processes nor any toxicological data specifically related to these components, when irradiated, were available to the Committee, it was unable to accept the suggested general extension of the safety assessment of irradiated foods to any foodstuffs irradiated at any dose as proposed by the WHO Expert Group of 1994 and the WHO Study Group on high-dose irradiation of 1997.

With respect to the irradiation of foods with doses above 10 kGy the additional studies on radiation chemistry have shown that, under the technological conditions necessary for obtaining irradiated foods of an acceptable organoleptic quality, chemiclearance, commonality of radiolytic reaction products and predictability of radiation chemical reaction mechanisms can continue to be used in the assessment of the safety of foods. 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. Again, only very limited toxicological studies have been carried out with foods irradiated with doses >10 kGy and none have been provided on any of the convenience foods which have been deep frozen and subsequently irradiated with doses above 10 kGy. As the existing toxicological database has been hardly extended 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.

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