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HIGH-DOSE IRRADIATION: WHOLESOMENESS OF FOOD IRRADIATED WITH DOSES ABOVE 10 kGy

Report of a
Joint FAO/IAEA/WHO Study Group



World Health Organization

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Joint FAO/IAEA/WHO Study Group on High-Dose Irradiation (Wholesomeness of Food Irradiated with Doses above 10 kGy)

Geneva, 15–20 September 1997

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1. Introduction

A Joint FAO/IAEA/WHO Study Group on High-Dose Irradiation met in Geneva from 15 to 20 September 1997. Dr F. S. Antezana, Deputy Director-General *ad interim*, opened the meeting on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations (FAO), the International Atomic Energy Agency (IAEA), and the World Health Organization (WHO). He said that the three Organizations had had a long and successful history of collaboration in the area of food irradiation, which had started as early as 1961. In 1980, the Joint Expert Committee on the Wholesomeness of Irradiated Food had concluded that the "... irradiation of any food commodity up to an overall average dose of 10 kGy¹ presents no toxicological hazard... and introduces no special nutritional or microbiological problems" (1). These conclusions clearly established the wholesomeness of any food irradiated up to an overall average dose of 10 kGy.

The reasons for this limitation to doses of up to 10 kGy were essentially two-fold. Firstly, the 1980 Joint Expert Committee was asked to assess the wholesomeness of irradiated foods on the basis of the data available at that time, which mainly concerned doses below 10 kGy. Secondly, many of the anticipated applications for irradiation of food would require doses of less than 10 kGy. Examples of such applications include: the elimination of vegetative bacterial pathogens from foods such as meat, poultry, fish, and fresh fruits and vegetables; the inhibition of sprouting in potatoes and other tubers; the insect disinfestation of grains and dried fruits such as dates and figs; extension of the shelf-life of refrigerated foods; and the treatment for quarantine purposes of fruits and vegetables. Although the Joint Expert Committee recognized that higher doses were needed for the treatment of certain foods, it did not undertake a toxicological evaluation or a wholesomeness assessment of food treated with higher doses, because the available data at that time were insufficient. It concluded that further studies in this area were required.

On the basis of the scientific judgement provided by the Joint Expert Committee in 1980, as well as additional supportive evidence, the FAO/WHO Codex Alimentarius Commission adopted, in 1983, the Codex General Standard for Irradiated Foods, limiting the overall average dose to 10 kGy (2). As a consequence, a large number of governments

¹ The gray (Gy) is the unit of absorbed dose of ionizing energy, and is equivalent to 1 joule/kg. The gray replaces the rad (radiation absorbed dose) as the unit of absorbed dose. One gray is equivalent to 100 rads.

(currently 40) initiated regulatory actions permitting the irradiation of a considerable number of food commodities.

With the exception of irradiation of spices and dried vegetable substances, which is widespread, other applications of this technology remain marginal. Misconceptions about whether irradiated food is safe to eat and about how irradiation can complement or replace other methods of preserving food are largely responsible for this situation. Consequently, the beneficial results of food irradiation – the improvement of the hygienic quality of certain foods and the reduction of post-harvest losses – are not generally available to individual consumers, families and societies. There are indications, however, that irradiation will be increasingly used to ensure hygienic quality of food of animal origin and to overcome quarantine barriers in trade in fresh fruits and vegetables. An outbreak of infection with enterohaemorrhagic *Escherichia coli* in the United States of America in August 1997 led to the recall of 25 million pounds (over 10 000 metric tonnes) of ground beef in which the contamination with this pathogen could not be excluded. Events of this kind make a case for the use of food irradiation as a public health technology. Moreover, the use of high-dose irradiation could also result in less dependence on refrigeration of food, which is an energy-intensive technology.

The fact that the international organizations and the Codex limited the dose level to 10 kGy has frequently been interpreted as meaning that this is a dose above which toxic substances could be introduced or nutritional adequacy of foods could be negatively influenced. However, there are current applications of food irradiation involving doses above 10 kGy which indicate that this is not the case. These include the development of high-quality shelf-stable convenience foods for general use and for specific target groups, such as immunosuppressed individuals and those under medical care. Such shelf-stable foods have also been used successfully by astronauts, military personnel and outdoor enthusiasts in some countries. The present Study Group was convened to evaluate the data that have become available on irradiation of foods with doses above 10 kGy in order to determine whether such foods can be considered as safe and nutritionally adequate.

1.1 Objectives of the Study Group

The objectives of the Study Group were:

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

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

1.2 Guiding principles

In evaluating the data relating to the high-dose irradiation process, the Study Group was guided by prior established principles for determining the wholesomeness of foods so processed: that such foods be deemed safe if they pose no toxicological or microbiological hazards; and that they be deemed adequate for consumption if they pose no special nutritional problem. The Study Group was further guided by the recommendations of the 1976 Joint FAO/IAEA/WHO Expert Committee that the determination of wholesomeness for a representative food could be extrapolated to other foods of similar composition on the basis of available chemical data (3). Consistent with these principles, the present meeting focused on the wholesomeness of foods or classes of foods (i.e. meats, fruits/vegetables), appropriately pretreated and packaged, that are irradiated to average doses higher than 10 kGy to reduce or eliminate pathogenic and spoilage microorganisms as may be required for the particular product.

There are several aspects of food irradiation in general and high-dose irradiation in particular that have to be considered in order to evaluate comprehensively the wholesomeness of foods treated by high doses of radiation. For example, the extension from low doses (less than 10 kGy) to high doses does not involve merely additional exposure, as low doses are normally associated with radiation pasteurization at chilled or ambient temperatures, while high doses are used on foods that are either dry or frozen. In many cases, the chemical consequences of irradiating with high doses at subfreezing temperatures are essentially equivalent to irradiating with low or moderate doses at chilled temperatures. For these reasons, considerations of the radiation effects described in this report place special emphasis on the conditions appropriate to high-dose applications, including low-temperature processing, an anoxic environment, and barrier packaging.

2. General considerations

2.1 Reasons for high-dose food irradiation

As mentioned in section 1, the Codex General Standard for Irradiated Foods, adopted in 1983 by the Codex Alimentarius Commission (2), limits the application of irradiation up to an *overall average dose* of 10 kGy. This decision of the Commission was based on recommenda-

tions contained in the report of the 1980 Joint Expert Committee (1). The reasons for this limitation are explained further in section 2.2.

The 1980 Joint Expert Committee did not see the 10 kGy limit as a major handicap to the practical use of food irradiation because, again as indicated in section 1, most of the anticipated applications would require doses below 10 kGy. Experience has shown, however, that this limit of not more than 10 kGy can cause certain difficulties. For example, most commercial radiation facilities operate in a way that produces a dose spread corresponding to a maximum to minimum dose ratio (D_{\max}/D_{\min}) of 2 to 3. This means that an irradiation run intended to treat the food to an overall average dose of 10 kGy would result in some material receiving a dose of only 5 kGy, which may not be enough to reduce or eliminate reliably certain pathogenic microorganisms in that material. An average dose higher than 10 kGy is needed to ensure the desired safety standard.

The presence in food of pathogenic microorganisms, such as *Salmonella* species, *Escherichia coli* O157:H7, *Listeria monocytogenes* or *Yersinia enterocolitica*, is a problem of growing concern to public health authorities all over the world. In an attempt to reduce or eliminate the resulting risks, measures such as strict hazard analysis critical control point (HACCP) system regulations have been issued in many countries. In the United States, for instance, the Department of Agriculture has issued regulations regarding the application of the HACCP system in the processing of raw meat and poultry products with the objective of preventing or minimizing contamination of these products. To ensure that these products are consistently free of pathogens, irradiation to a dose of 10 kGy or less could be considered a Critical Control Point in the HACCP plan for these products. In some instances, however, an upper limit of 10 kGy for the overall average dose could preclude the effective use of this method.

In the case of spice irradiation, this need for a higher average dose has already been recognized in several countries. France permits an average dose of 11 kGy for the irradiation of spices and dry aromatic substances, and Argentina and the United States permit a maximum dose of 30 kGy (D_{\max}) for this purpose.

Still higher doses are required for radiation sterilization of food, for instance, for immuno-compromised hospital patients. For this purpose, the Netherlands permits an average dose of 75 kGy. Some other countries, such as the United Kingdom, permit radiation sterilization of hospital diets, but have not specified a radiation dose limit for this application. South Africa has permitted the marketing of shelf-stable meat products irradiated to a minimum dose of 45 kGy, and

considerable quantities of such products have been marketed in recent years. Clearly, there are technological reasons for the use of radiation doses higher than 10 kGy.

The increasing cost of energy will probably increase the cost of producing and distributing foods, especially those of animal origin. Because of the high cost of refrigerated and frozen storage, developing countries in particular would benefit from the availability of wholesome foods with a prolonged shelf-life that do not require the use of this technology. Experience in South Africa has shown that irradiation in combination with other processes can provide shelf-stable food products of high quality that can be distributed easily under subtropical and tropical conditions with an energy expenditure much lower than that required for frozen storage.

2.2 **History of wholesomeness determination of irradiated food**

Extensive animal feeding studies designed to detect any toxic factors that might be present in various irradiated foods were carried out in the 1950s and 1960s, mostly in the United Kingdom and the United States. On the basis of these studies a Working Party established by the United Kingdom Ministry of Health agreed that extensive tests on a wide range of foods, carried out particularly in the United States, had yielded no evidence for the formation of carcinogens in irradiated food. The Working Party, after considering the effects of irradiation on nutrients, on the possible presence of induced radioactivity, and on possible microbiological hazards of irradiated food, also concluded “that the evidence for the wholesomeness of food which has been irradiated under specified and closely controlled conditions is reassuring” (4). The United States Army Surgeon General concluded in 1965 that “foods irradiated up to an absorbed dose of 5.6 Mrad (56 kGy) with a cobalt-60 source of gamma radiation or with electrons with energies up to 10 million electron volts (MeV) have been found to be wholesome, i.e. safe and nutritionally adequate” (5).

At about that time, however, the United States Food and Drug Administration and other national health agencies began to apply more stringent criteria for safety testing. Evidence from animal feeding studies found acceptable in the 1950s was considered to be insufficient. In response, a massive programme to test the safety of radiation-sterilized beef and, a few years later, of radiation-sterilized chicken meat was initiated in the United States.

The first international meeting exclusively devoted to a discussion of wholesomeness data and legislative aspects of irradiated foods was held in Brussels in October 1961. It was organized by FAO, IAEA and WHO

and was attended by participants from 28 countries. Although a delegate from the United States reported that long-term toxicity studies had been conducted on 22 representative foods, and participants from many other countries presented the results of other such studies, the meeting decided that general authorization of the commercial use of radiation for the treatment of food was premature. It was recommended that FAO, IAEA and WHO should consider the early establishment of a Joint Expert Committee to advise on the special requirements for the testing of the wholesomeness of irradiated foods (6).

The Joint FAO/IAEA/WHO Expert Committee on the Technical Basis for Legislation on Irradiated Food met in Rome in April 1964. The Committee stated that

extensive tests conducted by feeding to animals, and to a lesser extent to human volunteers, irradiated food treated in accordance with procedures that should be followed in approved practice have given no indication of adverse effects of any kind, and there has been no evidence that the nutritional value of irradiated food is affected in any important way (7).

The Committee recommended legal control of irradiated food “by the use of a list of permitted foods irradiated under specific conditions” and made recommendations as to which tests should be applied to an irradiated food to establish its safety for consumption; it suggested that these tests should be broadly similar to those used for testing the safety of food additives.

When it met in Geneva in April 1969, the Joint FAO/IAEA/WHO Expert Committee on the Wholesomeness of Irradiated Food gave “temporary acceptance” to irradiated potatoes (doses up to 0.15 kGy) and to wheat and wheat products (up to 0.75 kGy), but found the available data on irradiated onions to be unsatisfactory for an evaluation (8). The acceptance for potatoes and wheat was designated as temporary because the available data were insufficient to fully establish safety; additional evidence within a specified period of time was required.

In order to coordinate and rationalize the various efforts around the world to test the safety of radiation-sterilized foods, the International Project in the Field of Food Irradiation was created in 1970. Under the sponsorship of FAO, IAEA and the Organisation for Economic Co-operation and Development (OECD), 24 countries pooled their resources to address related issues. WHO became associated in an advisory capacity. Feeding studies contracted by the International Project were carried out with irradiated wheat flour, potatoes, rice, iced ocean fish, mangoes, spices, dried dates and cocoa powder. In view of the

extensive studies on high-dose irradiated products undertaken in the United States, the International Project limited its studies to the dose range up to 10 kGy. The International Project was terminated in 1982, when the Member countries found that it had fulfilled its purpose, clearly answering the question of wholesomeness of foods irradiated to doses not exceeding 10 kGy.

At its meeting in September 1976 in Geneva, the Joint Expert Committee gave “unconditional acceptance” to irradiated wheat (up to 1 kGy), potatoes (up to 0.15 kGy), papayas (up to 1 kGy), strawberries (up to 3 kGy), and chicken (up to 7 kGy), while onions (up to 0.15 kGy), rice (up to 1 kGy) and fresh cod and red-fish (up to 2.2 kGy) received “provisional acceptance”. The latter category meant – as did the previously used term “temporary acceptance” – that some additional testing was required. The Committee also considered irradiated mushrooms, but found that an evaluation was not possible with the available data (3).

The Committee gave much thought to the principles of testing the wholesomeness of irradiated foods and stressed the differences from the safety evaluation of food additives. It clearly defined irradiation as a physical process for treating foods and, as such, one comparable to the heating or freezing of foods for preservation; it also recognized the value of chemical studies as a basis for evaluating the wholesomeness of irradiated foods.

When the Joint Expert Committee held its following meeting, in Geneva in October 1980, it was provided with a wealth of additional data, mostly by the International Project. On this basis the Committee, in what represented a landmark report (1), came to the following conclusions:

- None of the toxicological studies carried out on a large number of individual foods (as representatives of different classes of food having similar chemical compositions) had produced evidence of adverse effects as a result of irradiation.
- Radiation chemistry studies had shown that the radiolytic products of major food components were identical, regardless of the food from which they were derived. Moreover, for major food components, most of these radiolytic products had also been identified in foods subjected to other, accepted types of food processing. Knowledge of the nature and concentration of these radiolytic products indicated that there was no evidence of a toxicological hazard.
- Supporting evidence was provided by the absence of any adverse effects resulting from the feeding of irradiated diets to laboratory animals, the use of irradiated feeds in livestock production, and the practice of maintaining immunologically incompetent patients on irradiated diets.

The Committee therefore concluded that irradiation of any food commodity up to an overall average dose of 10 kGy presented no toxicological hazard; hence, toxicological testing of foods so treated was no longer required.

The Committee further concluded that the irradiation of food up to an overall average dose of 10 kGy introduced no special nutritional or microbiological problems. However, it emphasized that attention should be given to the significance of any changes in a particular irradiated food in relation to its role in the diet.

The Committee recognized that higher doses of radiation were needed for the treatment of certain foods but considered that the available data were insufficient for a toxicological evaluation and wholesomeness assessment of food so treated and that further studies in this area were needed. The final results of the studies carried out in the United States on high-dose irradiated food items were not available at that time.

At the request of the Australian Ministry for Community Services and Health, WHO subsequently commissioned an updated, comprehensive analysis of the safety and nutritional adequacy of irradiated food. An *ad hoc* group of experts, invited by WHO, reviewed and evaluated scientific studies conducted after the 1980 Joint Expert Committee meeting, including studies on the high-dose (59 kGy) irradiation of chicken carried out in the United States (9), as well as many of the older studies that had already been considered previously. The report of this evaluation was published by WHO (10). The group concluded that irradiated food produced under established good manufacturing practice (GMP) could be considered safe and nutritionally adequate because the process of irradiation:

- would not lead to changes in the composition of the food that, from a toxicological point of view, would have an adverse effect on human health;
- would not lead to changes in the microflora of food that would increase the microbiological risk to the consumer;
- would not lead to nutrient losses to an extent that would have an adverse effect on the nutritional status of individuals or populations.

The International Consultative Group on Food Irradiation (ICGFI) decided in 1989 to assemble, with the help of consultants and in collaboration with WHO, all relevant data on radiation applications involving doses above 10 kGy to determine whether or not the information available would be adequate for an assessment of the wholesomeness of food irradiated to these doses. On the basis of reports written by several experts, a Consultation was held in Karlsruhe in 1994.

In its report, the Consultation concluded that the available data on radiation chemistry, toxicology, microbiology and nutritional properties of food were adequate for this purpose (11).

3. **Radiation chemistry considerations**

3.1 **Introduction**

As with other food processes such as pasteurization and sterilization technologies involving the input of thermal, mechanical or photonic energy, the objective of processing with ionizing radiation is to destroy pathogenic and spoilage microorganisms without compromising the safety, nutritional properties and sensory quality of the food. All these processes produce physical and chemical changes, but the extent of these changes differs significantly. Depending on the type of energy, its penetration into the food, and the amount of energy ultimately deposited, several different chemical bonds in the constituents are broken or formed, leading to either desired or undesired effects. In comparison to thermally sterilized foods, the extent of chemical change in radiation-sterilized foods is relatively small and uniform. It is through a consideration of the radiation chemistry of food (12–14) that these chemical differences and their implications for wholesomeness and product quality can be understood.

3.1.1 ***Relation to efficacy, wholesomeness and sensory attributes***

Once the physical processes by which ionizing radiation loses energy to atoms constituting the food have been completed, it is the resultant formation and reaction of specific chemical entities that ultimately determine the destruction of contaminating microorganisms, the potential formation of a toxic compound, the retention of micronutrients, the retention of sensory attributes, and even the retention of package functionality. Microorganisms are destroyed primarily because hydroxyl radicals formed within their cells react with the base and sugar moieties of DNA; this results in part in breakage of sugar–phosphate bonds and loss of the replication function. A compound capable of eliciting a chronic toxic or genotoxic response can only be formed at a relevant level if a pathway for its formation is possible in principle and competitive in practice. Micronutrients, in particular vitamins, will be degraded to an extent that will depend both upon their ability to compete against other major constituents for the primary radicals, and upon the irradiation conditions, including dose. Sensory attributes, such as flavour, colour and texture, will similarly be affected if the constituents normally associated with these attributes can effectively compete for the

primary radicals and then follow a reaction pathway that leads to a stable product with different sensory characteristics. Package functionality might be favourably or unfavourably affected by the competition between bond-breaking and bond-making reactions, which are influenced by the chemical structure of the material and irradiation conditions. In summary, the consequences to the microorganisms, to the food constituents and to the packaging are determined by well-established principles of radiation chemistry.

3.1.2 **Relevance to high dose**

An understanding of the chemistry involved is especially relevant to the assessment of the safety and applicability of using high-dose irradiation to sterilize foods and render them shelf-stable. It explains the commonality in the chemical and microbiological consequences between high-dose and low-dose applications, which primarily involve pasteurization, improved sanitation, and enhanced shelf-life, and it provides the rationale for delivering high doses either to dry foods at room temperature or to enzyme-inactivated, high-moisture muscle foods at subfreezing temperatures. The assessment to be made is, in principle, a consideration of the nature and extent of chemical change in the irradiated foods and of the impact these changes would have on the health of individuals consuming such foods. If the radiolytic mechanisms by which food constituents undergo certain transformations, the dependence of radiolysis products on absorbed dose, and the influence of processing conditions on product yields are all known, it is possible to make a valid extrapolation of the results and conclusions from one particular food to a class of foods, from one dose regime to another, and from a particular set of conditions to another applicable set (15).

3.1.3 **Aim**

The aim of this section is to show that: (a) the overall extent of chemical change in the food constituents is comparatively low and in principle calculable; (b) the nature of such changes is common to similar foods and generally predictable on the basis of composition and irradiation conditions; (c) there is a significant reduction in the overall chemical change in constituents associated primarily with the aqueous phase when the food is irradiated while frozen; and (d) data pertaining to the safety or functionality of irradiated foods can be validly extrapolated from one food system to another.

3.2 **Basic principles**

The primary chemical entities formed in an irradiated matrix and ultimately involved in reactions leading to stable radiolysis compounds

are a consequence of complex physical and physicochemical processes that start with localized interaction of the radiation with constituent atoms and continue to the point where these entities are uniformly distributed and react in conformity with the principles of homogeneous kinetics (16). The interaction between the atoms and fast-moving high-energy electrons, introduced directly or generated from gamma-rays or X-rays through either the photoelectric or the Compton process, results in the absorption of energy and the consequent ionization and excitation of constituent molecules. This energy deposition process occurs within 10^{-16} s. Many high-energy processes then ensue, including energy migration and ion-molecule reactions; many relaxation and thermalization processes take place, including electron solvation; and some reactions occur simultaneously with diffusion away from the site of initial formation. These processes occur within about 10^{-11} s. Subsequently, the more stable but nevertheless reactive entities in thermal equilibrium with the matrix begin to diffuse out and react, primarily with each other, but also with solutes present at high concentration. These further processes, which lead to a relatively uniform distribution of radicals, occur within about 10^{-7} – 10^{-6} s. The formation of these stable entities and reactive radicals can be thought of as the “direct effect”. Subsequently, the fate of the precursor radicals, the yields of which in pure systems have been generally determined, can be altered through reaction with minor constituents. The formation of stable radiolysis products through these reactions can be thought of as an “indirect effect”. The specific nature of the primary chemical entities formed initially and the precise amount of them that might become uniformly distributed depend on the molecular nature of the matrix.

3.2.1 **Radiolysis of water**

Since water constitutes about 65% of the mass of the muscle foods likely to be sterilized by irradiation and, since it contains many dissolved solutes of interest, its radiolysis is of particular interest (17, 18). When water is irradiated, the ionization produces an energetic electron and a cation radical, while excitation produces an excited water molecule. The ejected electron, after losing energy and reaching thermal equilibrium with the surrounding water molecules, can be trapped by a favourable configuration of water molecules to produce a solvated electron, e_{s}^{-} , or can be drawn back to the cation, the ensuing neutralization reaction producing an excited water molecule. The solvated electron is a highly mobile, highly reducing primary entity (18–20); it is a precursor of many secondary entities. The excited water molecule can either lose its excess energy or dissociate into two other primary entities: hydrogen atoms (H^{\bullet}) and hydroxyl radicals (OH^{\bullet}); both are highly mobile, the former being a

strong reductant, the latter a strong oxidant. The cation radical is extremely short-lived, its major pathway for reaction being proton transfer to water, producing the hydronium ion (H_3O^+) and OH^\bullet . Various recombination and cross-combination reactions of the primary radicals are possible, the combination of H^\bullet and OH^\bullet regenerating water (H_2O). Such reactions occur simultaneously with diffusion away from the sites of energy deposition, which results in a specific number of primary radicals being distributed throughout the medium.

G-value

For purposes of accounting for all of the processes relating to the formation and distribution of primary chemical entities and for all of the consequent reactions leading to secondary entities and final stable products, the yield of these entities is given in terms of their *G*-value. As defined in much of the literature, it is the number of entities (including transient entities or stable compounds) either formed or lost for every 100 eV of absorbed dose. (The newer SI-based definition is given in terms of moles per joule (mol/J); converting from the original definition involves multiplying by 1.04×10^{-7} .) For neutral water subjected to the kinds of radiation permitted for use with food, the relevant *G*-values (using the older definition) for e_s^- , OH^\bullet , H^\bullet , molecular hydrogen (H_2) and hydrogen peroxide (H_2O_2) are 2.7, 2.7, 0.6, 0.4 and 0.7, respectively (18, 21). They indicate the predominance of e_s^- and OH^\bullet as precursors and the fixed formation of molecular hydrogen and hydrogen peroxide. Of particular relevance is the reactivity of the primary radicals.

Typical radical reactions

The possible reactions of the primary radicals with dissolved solutes include: abstraction, addition and oxidation/reduction (18, 22). *Abstraction* can be thought of as transferring a hydrogen atom from a weak and accessible C–H bond to form a strong H–H or H–OH bond. Since bond-breaking is involved, the most likely site of abstraction will be at the weakest C–H bond in the molecule and the rate constant will have a finite activation energy. *Addition* of small radicals to double bonds, especially in aromatic or heterocyclic rings, is an energetically very favourable reaction that is essentially a diffusion-controlled process, the rate constants being very high. It tends to be nonselective, so all accessible multiple bond sites, including C=N and C=C, are about equally affected. The solvated electron can also add to aromatic and heterocyclic rings, as well as to the C=O group. Electron addition followed by dissociation also occurs and is favoured when the substituent has a high electron affinity. *Oxidation and reduction* reactions

involve the transfer of an electron from a donor to an acceptor with accompanying changes in charge and valence state. The redox potentials of the reacting partners determine the direction of the electron transfer and influence the rate constants. A wide variety of inorganic cations and anions as well as organic molecules can be oxidized or reduced by the primary radicals. In all cases, the resultant secondary radicals can also be involved in subsequent abstraction, addition and redox reactions, the reaction rates being influenced by steric and energetic factors. Some of these reactions will lead to stable final products, while others will produce tertiary radicals that could combine to form stable products.

Typical product yields

The ultimate effect of the formation and reaction of primary radicals is to produce a net chemical change, which can be put into perspective by considering *G*-values and total absorbed dose. Accordingly, the concentration of a particular product, *P*, formed by the reaction of a solute with either e_s^- or OH^\bullet in a fluid solution irradiated to an absorbed dose of 4.5 kGy is estimated as 1.2 mmol/l, using the expression:

$$[P] = 0.1(G\text{-value})(\text{dose, in kGy})$$

Compared to the enormous changes that take place in heat-treated foods, this maximum yield due to a major precursor is extremely small. As these straightforward calculations indicate, it is possible to estimate not only the yield of products from any particular precursor, but the maximum yield of all derived products at any dose.

3.2.2 Irradiation parameter effects

Irradiation parameters, including the composition of the atmosphere in contact with the food, the temperature and phase of the food, the rate at which the dose is delivered and the total absorbed dose, can influence the direction and extent of the reactions by which primary and secondary chemical entities form stable products (23, 24).

Atmosphere

The presence or absence of oxygen (O_2) in the head space can influence the chemistry by introducing new pathways for reaction. Because of its high electron affinity, O_2 reacts readily with e_s^- and H^\bullet and with organic radicals. Reaction with the former leads to the formation of $O_2^{\bullet -}$ or HO_2^\bullet , which react primarily to yield H_2O_2 . Reaction with organic radicals leads to the formation of RO_2^\bullet radicals, which tend to react bimolecularly to form peroxides, but can also decompose unimolecularly to R^\bullet and HO_2^\bullet . This pathway is particularly relevant to the reaction in which a hydroxyl radical adds to an aromatic ring forming a further radical; in the presence

of oxygen, this results in simple hydroxylation (25), as in the conversion of phenylalanine to tyrosine. Reactions with lipid radicals can involve subsequent hydrogen abstraction, resulting in another radical and a hydroperoxide.

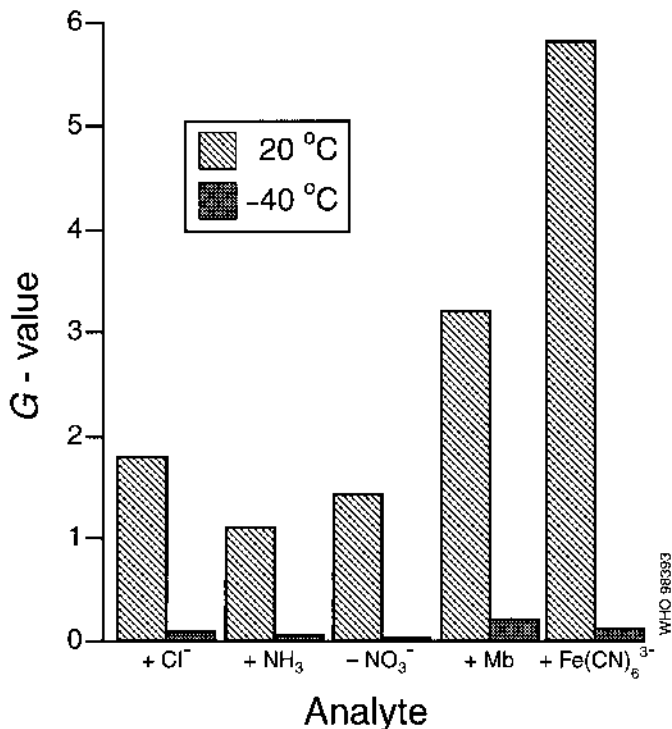
Temperature/phase

Because many radical reactions proceed with very low activation energies, changes in temperature only slightly increase or decrease the rate constants. However, if there are two competing reaction pathways with different activation energies, the temperature may influence the direction taken, depending on the extent to which it affects the rate constants.

Phase changes can have a substantial influence on the outcome of the radiolysis, primarily as a result of changes in the mobility of the constituent molecules and of any reactive entities derived from them.

Figure 1

Comparison of G-values resulting from irradiation in liquid solutions at 20 °C and in frozen solutions at -40 °C; the abscissa indicates the formation (+) or loss (-) of the indicated analyte upon reaction of different radicals with specific solutes^a



^a Reproduced from Taub et al. (27) with the permission of the publisher.

Phase affects the formation and distribution of primary radicals as well as their subsequent reactions with and within the confining matrix. For example, in frozen water the yield of primary radicals is substantially reduced (26), and these radicals tend to react either with each other or with major constituents in their proximity rather than with low concentrations of solutes that are constrained from diffusing and are separated by long intermolecular distances. This effect is illustrated in Fig. 1, which compares the formation or loss of a product analyte in a liquid solution at 20 °C with a frozen solution at -40 °C (27). The first four histograms from the left correspond to reactions of e_s^- with solutes, including the nitrate ion; the histogram on the right corresponds to the oxidation of the ferrocyanide ion by OH^\bullet , whose yield is doubled by the reaction of e_s^- with N_2O . This comparison illustrates the rationale for using frozen (or dry solid) foods when applying high sterilizing doses of irradiation; it also helps to explain why the extent of chemical change in such foods is quantitatively not unlike that observed in chilled foods receiving lower pasteurizing irradiation doses.

Because molecular relaxation and diffusion processes in solids can be influenced by temperature, the extent of certain reactions in frozen aqueous solutions can be strongly temperature dependent. In the formation of nitrite by the reaction of e_s^- with nitrate in frozen solutions irradiated at temperatures of -100 to -10 °C, there is only a small increase in yield with increasing temperature until about -30 °C, but thereafter it rises sharply with temperature; the ratio of the *G*-value at -10 °C to the *G*-value at -30 °C being about 10. The same effect is seen with other indices of reaction, such as reduction of the brown ferrimyoglobin by e_s^- to the red ferromyoglobin. Here too, the rationale for irradiating frozen food starting at a temperature of -40 °C can be understood.

Dose rate

The rate at which energy is deposited influences the rate of increase in the concentration of reactive radicals, which could have an influence on their reaction pathways (23–25). If there is the possibility of competing bimolecular and unimolecular (or pseudo-unimolecular) reactions, then the bimolecular process is favoured at high radical concentrations. Accordingly, in the following competition between bimolecular combination and unimolecular dissociation of an acyl radical,



the likelihood of reaction (1) increases with a substantial increase in dose rate, since the concentration of $RCH_2C^\bullet O$ will be higher at any one time.

Dose dependence

In general the yield of a particular radiolysis product will increase linearly with dose, although there can be deviations from such linearity, depending on the range of doses used. If the precursor of the product is a major food constituent and the dose is insufficient to result in a product concentration capable of competing for primary chemical entities, then the yield of that initial product will remain linearly dependent on dose. At high doses, however, some products with high reactivities will reach high enough concentrations to compete, which will result in their yields remaining constant and the yields of secondary products derived from them increasing linearly with increasing dose. If the precursor of a particular product is a minor constituent in the food, then the yield of that product will increase until the precursor is depleted and then remain constant thereafter, providing it is not reactive towards primary entities. A product of a minor constituent that is capable of competing for primary entities will eventually be depleted, resulting in the formation of a secondary product. The different possible yield–dose relationships have been discussed elsewhere (15), and the relevance of such dependencies to the validity of extrapolating safety data from one dose range to another was considered and illustrated. Since energy deposition is partitioned according to the mass fraction of the components, major radiolysis products are expected to be derived from the major constituents – water, proteins, lipids and carbohydrates – and to be formed in yields that are linearly dependent on dose in the practical range anticipated for radiation sterilization (23).

3.3 Major constituents

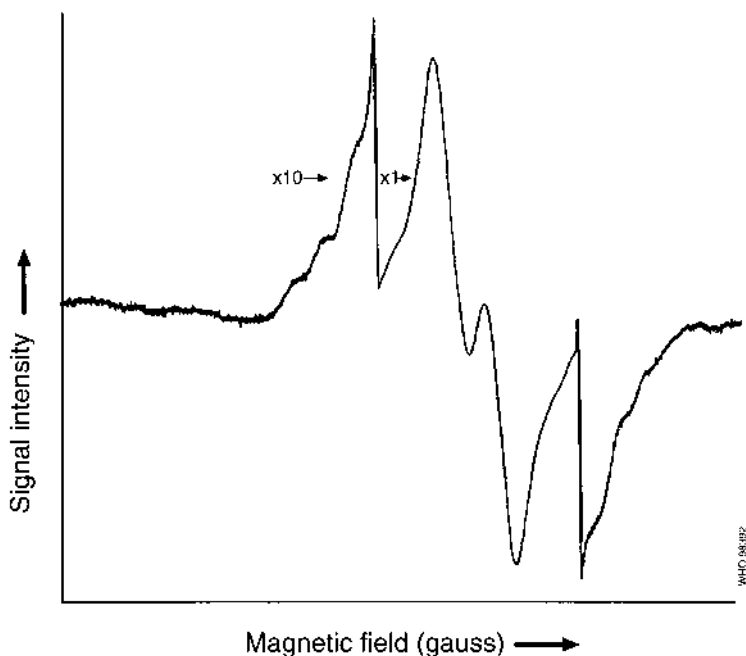
The radiolysis of major constituents in as complex a matrix as a muscle food can be understood by considering each constituent separately, since the chemistry tends to be compartmentalized. In chilled and, especially, in frozen muscle foods, the deposition of energy and the consequent chemical reactions occur over short ranges within almost distinct and immiscible phases. The main constituent, water (65%), surrounds and to differing extents suffuses the other two major constituents, proteins (20%) and lipids (15%). The water extensively suffuses the proteinaceous myofibrils comprising primarily myosin and actin. The depot fat comprising different triglycerides is essentially separate. There are many interfaces between the fat and the other constituents, but interfacial reactions are not expected to be significant. Each constituent phase contains soluble materials: the water contains sarcoplasmic proteins, including myoglobin and albumin, as well as diverse vitamins, salts and small peptides; the proteins can bind certain compounds, including thiamine; and the fat contains vitamins A and E, as well as other

compounds. Although carbohydrates make up only a small fraction of muscle tissue, they are important dietary components and constitute a large fraction of other foods (e.g. vegetables and bread) that might be irradiated together with meats. The basic radiolysis of the proteins, lipids and carbohydrates constituting the macronutrients of muscle foods can be considered within this framework.

3.3.1 **Proteins**

The major consequence of the ionization and excitation processes accompanying energy deposition in proteins is the formation of a peptide backbone radical, corresponding to scission of the backbone C–H bond (27, 28). Proof of this formation is given by the electron spin resonance (ESR) spectrum obtained upon irradiating a suspension of myosin/actomyosin, which shows a broad asymmetric doublet (Fig. 2). Spectral analysis, based on spectra obtained by irradiating diverse dipeptides, indicates that the doublet is a composite of many spectra in which the unpaired electron interacts primarily with a single proton bound to the carbon atom linking the sidechain moieties of constituent amino acids to the peptide backbone (28). The spectrum also shows a much less intense

Figure 2
Electron spin resonance spectrum of suspended myosin/actomyosin irradiated to 60 kGy at -40°C and scanned at 77 K^{a}



^a Reproduced from Taub et al. (28) with the permission of the publisher.

contribution of radicals corresponding to H^{\bullet} addition to the benzene ring of aromatic amino acid moieties. The ESR spectrum produced by irradiating the suspension of the myofibril bundles or the whole muscle is the same (29), indicating that the aggregation of myosin into more complex structures does not affect the mechanism of radical formation, which occurs on a molecular scale.

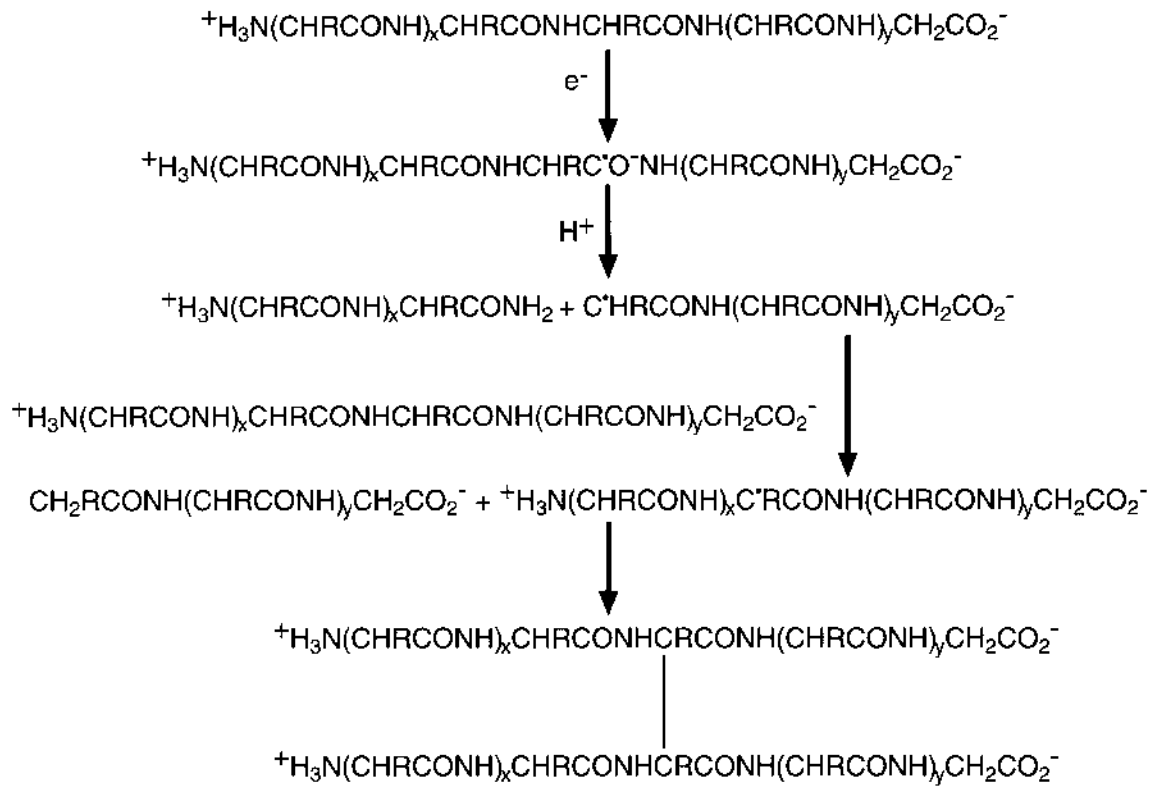
Although the radiolysis of proteins is analogous to the radiolysis of water, the formation of the peptide radicals (and other reactive entities) and the pathways for their subsequent reaction are all understandably more complex (27). The sequence of reactions initiated by electron attachment to the peptide carbonyl group shown in Fig. 3 not only illustrates some of this complexity, but also highlights some of the preferred steps that will be common to all proteins. The first step leads to an observable carbonyl anion radical that dissociates into a stable amide and an alkyl radical; another step involves the abstraction of hydrogen from the C–H backbone by this radical to form a stable compound and the peptide radical; the last step is the bimolecular reaction of this radical, either to dimerize, forming a cross-linked myosin, or to disproportionate, reforming the myosin and forming an imine with its hydrolysable $N=C$ linkage. Despite the size of the peptide radical, it will react at $-10^{\circ}C$ over the course of hours; upon thawing, it will disappear rapidly.

Sequences of reactions initiated by H^{\bullet} and OH^{\bullet} will differ from that initiated by the solvated electron, but some of the same intermediates will be formed (28). H^{\bullet} could react at the backbone C–H and at the carbonyl group, but is more likely to add to aromatic or heterocyclic rings in the sidechain moieties. OH^{\bullet} could react at the backbone C–H, but is also more likely to add to side groups. The relatively high rate constants for addition indicate nonselectivity, so all accessible ring amino acid moieties are equally likely as reaction sites. As a consequence of the formation of these addition radicals, there is the possibility of formation of cross-linked proteins through the sidechains. Studies with peptides of phenylalanine demonstrate how OH^{\bullet} is involved in such cross-linking and how cross-linked products can be found without the initially added OH^{\bullet} (30, 31). Other less predominant processes can occur, including some sidechain scission leading to low, but observable levels of volatiles derived from specific amino acid moieties (32).

The implication from this mechanistic understanding is that proteins in irradiated frozen meats would be slightly altered by some aggregation and fragmentation, to an extent limited by the low G -values for primary radical formation. Moreover, there would be only slight discrimination among the amino acid moieties affected. Experimental measurements,

Figure 3

Mechanistic scheme for reactions in proteins initiated by solvated electron addition to the carbonyl group in the peptide backbone



using electrophoresis to assess changes in protein molecular size, enzymatic hydrolysis to assess digestibility, and amino acid analysis of acid-hydrolysed samples, bear this out. In particular, within the limits of sensitivity for analysing amino acids, there is no dose-related change in amino acid composition over the dose range anticipated for use in sterilization (23).

3.3.2 *Metalloproteins*

The presence within a protein molecule of a metal ion that can be oxidized or reduced provides additional pathways for reaction of both primary and secondary entities. This potential for modifying the radiation chemistry is especially significant for small or globular proteins, such as the pigment myoglobin. Because the metal ions represent such a small proportion of the total host protein mass and are fixed in specific locations within the molecular geometry, they can only influence the reaction of primary entities or small secondary radicals when at exposed and accessible sites, and their influence on the fate of radicals formed in the host protein is limited to relatively short-ranged reactions. Nevertheless, the reactions are distinctive and have been studied in detail in both model and food systems (33–39).

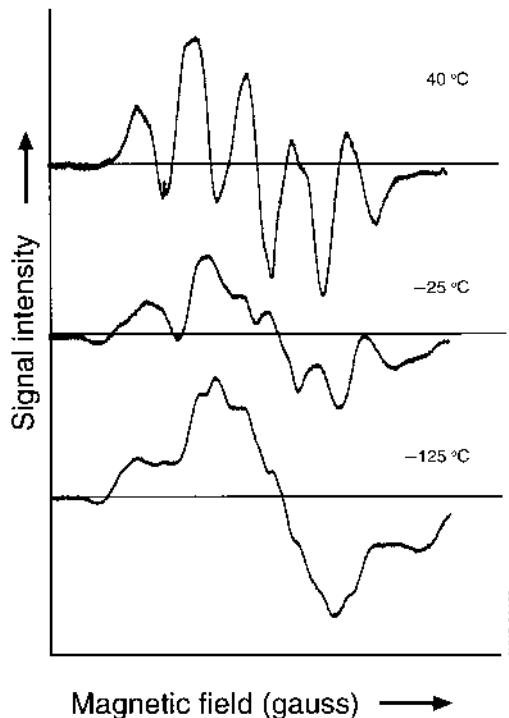
The radiolysis of myoglobin, which is very relevant to irradiated red meats, is illustrative. In this case, the iron ion is centred in the planar haeme group and forms a complex at one apical coordination site with a histidine moiety from one α -helix and at the other apical site to one of several possible molecules including water, oxygen or nitric oxide. Studies in dilute aqueous solution show a complex series of reactions between e_s^- or OH^\bullet and ferromyoglobin (Fe^{2+}), ferrimyoglobin (Fe^{3+}) or oxymyoglobin, the course of reactions being influenced by oxygen and hydrogen peroxide. Under carefully controlled conditions, certain reactions can be followed separately, such as the reduction of ferrimyoglobin to ferromyoglobin. The reactions of e_s^- , H^\bullet and OH^\bullet follow a generally predictable course appropriate to proteins. Accordingly, e_s^- can react with the peptide bond, and all of the primary entities can add to the ring groups of the aromatic and heterocyclic amino acid moieties.

There are implications here for both the primary reactions and the secondary radicals. The high reactivity of Fe^{3+} for e_s^- and of Fe^{2+} for OH^\bullet would tend to reduce the G -values of products traceable to these precursors. Moreover, because the iron is capable of reacting intramolecularly with some radicals formed in the myoglobin, intermolecular cross-linking is reduced and some restoration of bonds initially broken can be facilitated.

3.3.3 Lipids

The major consequences of the ionization and excitation of the triglycerides constituting the lipids in foods are the disruption of the bond between the fatty acid and glycerol moieties and the formation of the dominant triglyceride radical corresponding to an unpaired electron on the carbon atom in the alpha-position relative to the carbonyl group (40). Aside from chemical evidence of stable products derived from this radical, there is proof of its formation from the ESR spectrum of irradiated tripalmitin powder, a representative saturated triglyceride, which at 40 °C shows an asymmetric quintet reflecting the interaction of the unpaired electron with hydrogen atoms on the same carbon atom and on the neighbouring carbon atom (Fig. 4). In a triglyceride with polyunsaturated fatty acid moieties, radicals would be formed by scission of a C–H bond near the unsaturated functional group such that hydrogen is lost from the weak C–H bond of the methylene group in the linoleic moiety. As in the

Figure 4
Sequential electron spin resonance spectra (recorded at 77 K) of powdered tripalmitin irradiated at -125 °C and annealed first at -25 °C and then at 40 °C^{a,b}



^a Reproduced from Taub (25) with the permission of the publisher.

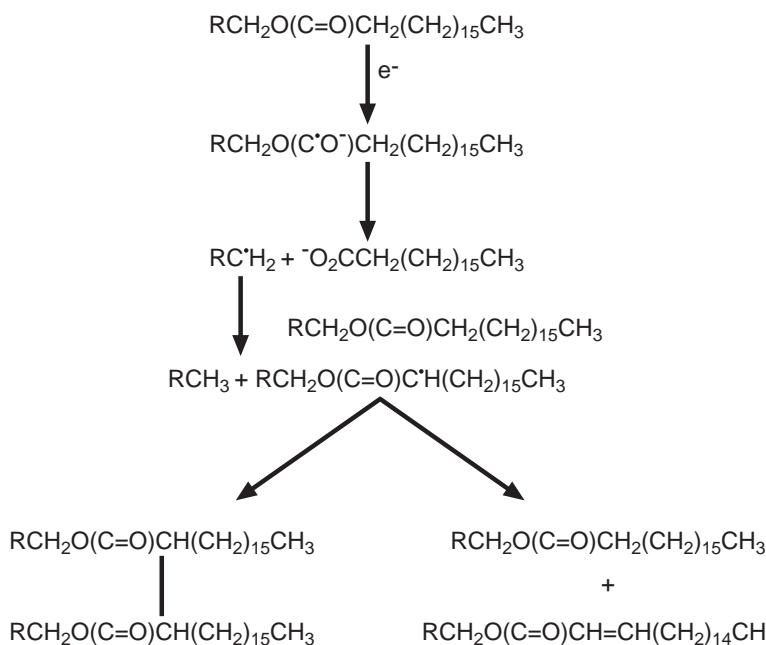
^b The spectra have been displaced vertically for clarity of comparison.

case of proteins, the molecular processes are independent of the way in which the triglycerides are organized, so the same radicals are observed in isolated systems and in a complex muscle food.

Coincidentally, there is considerable similarity in the sequence of reactions that lead to the most stable radical in lipids and proteins (24, 29). The sequence initiated in tripalmitin by electron reaction is illustrated in Fig. 5. It starts with electron attachment to the carbonyl group forming the carbonyl anion radical, whose broad singlet ESR spectrum can be observed at low temperature (-125°C). This radical then dissociates into the stable palmitic acid anion and an alkyl radical that then abstracts a hydrogen from the carbon alpha to the carbonyl group on another tripalmitin molecule forming the main radical, represented here by $\text{RCH}_2\text{O}(\text{C}=\text{O})\text{C}^{\bullet}\text{H}(\text{CH}_2)_{15}\text{CH}_3$. This large, slowly diffusing radical can react either by combination, forming a stable dimer, or by disproportionation, reforming the original tripalmitin and forming an unsaturated analogue. *G*-value measurements for irradiated tripalmitin range from 1.6 for both palmitic acid and molecular hydrogen to 0.6 for pentadecane, to 0.12 for the dimer and to 0.04 for palmitylaldehyde.

Figure 5

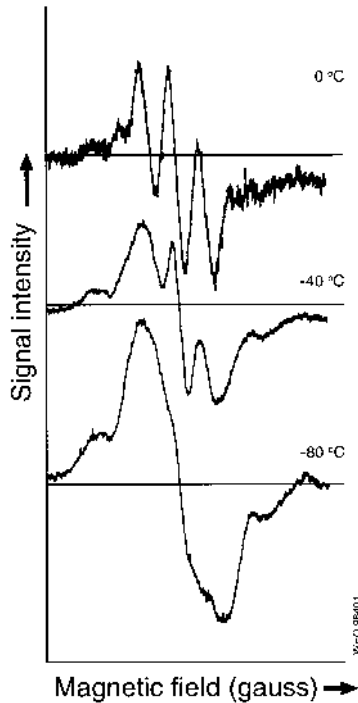
Mechanistic scheme for reactions in triglycerides initiated by electron addition to the carbonyl group near the ester linkage



WHO 9638B

Figure 6

Sequential electron spin resonance spectra (recorded at 77 K) of beef fat irradiated at -80°C and annealed first at -40°C and then at 0°C ^a



^a Reproduced from Merritt and Taub (59) with permission.

The implication for other triglycerides in complex muscle foods is that the same reactions will take place and a similar distribution of products corresponding to the constituent fatty acid composition will be observed. As Fig. 6 shows, a sequential formation and conversion of radicals is observed in irradiated beef fat leading to the preferred triglyceride radical at 0°C . Moreover, the net chemical change will be small, with products such as the fatty acid, hydrogen and the propanedioldiesters (derived from the initial alkyl radical) predominating. Much smaller yields of volatile compounds have been determined, and these provide very useful insights into the common pathways for reaction.

An especially low yield of 2-dodecylcyclobutanone (DCB), which can be derived from palmitic acid, has been detected in certain irradiated foods. The alkylcyclobutanones appear to be specific to the irradiation process, since they have not as yet been found in non-irradiated samples (41, 42). They are formed at about $0.5\ \mu\text{g}$ per gram of lipid at 5 kGy. It is possible that in lipids subjected to high temperatures in non-irradiated food these cyclic compounds are both produced and decomposed, so their residual concentrations would be quite low.

3.3.4 *Carbohydrates*

The major consequences of directly ionizing and exciting a carbohydrate molecule, such as starch, or of primary entities reacting with soluble monosaccharides or polysaccharides, such as glucose or sucrose, are the breaking of C–H bonds and the disruption of ether linkages (43–45).

In solids such as starches, bond breakage is mainly at the glucosidic linkage, leading to depolymerization and, eventually, to radicals centred on the C-1 and C-6 positions. Moreover, radicals formed in starches of different origins are identical, as evidenced by the ESR spectra of the two main radicals and the influence of water content (2.8–7.9%) on the rates of their disappearance during storage (up to 1 year after irradiation) (46). The results are qualitatively the same when the irradiation is carried out with or without oxygen present and at either room temperature or at 77 K. These same radicals are formed in maltotriose and glucose oligomers, and the influence of water content and storage time on their disappearance is the same (47).

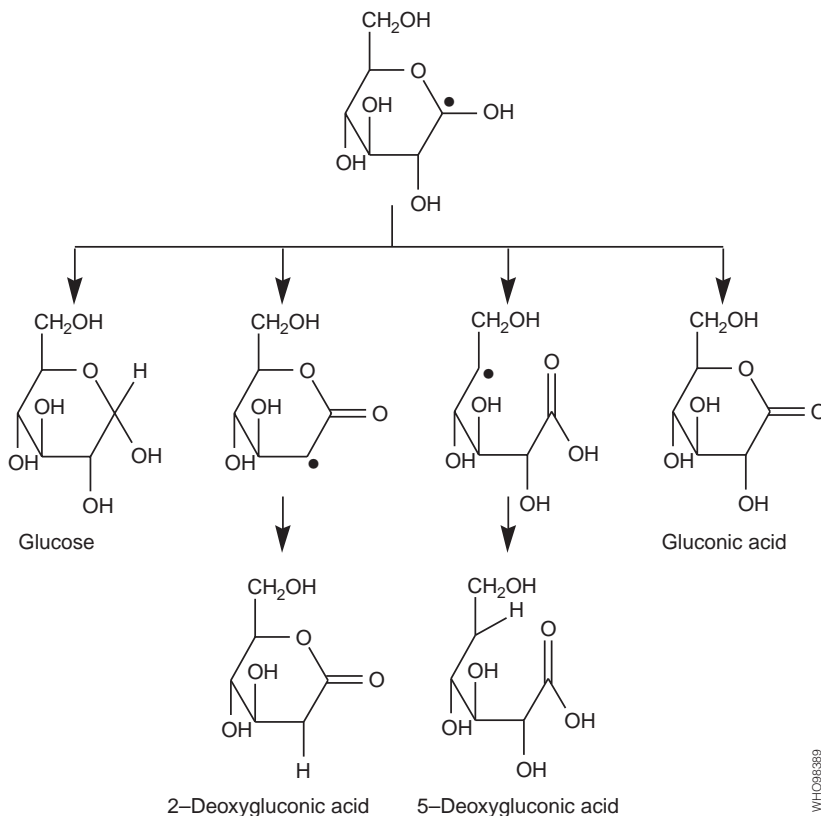
The results for radiolysis products formed in starches and their oligomers are also the same (47, 48). The quantities formed are proportional to the dose up to a value depending on the products concerned: 15 kGy for formic acid, 40 kGy for malonaldehyde, 50 kGy for dihydroxyacetone and glyceraldehyde, and 70–80 kGy for soluble dextrans (47–49). It is also possible to calculate the yield of soluble dextrans or the degree of depolymerization of the starch macromolecule knowing the characteristics of the initial starch, its water content and the irradiation dose (50, 51).

In solution, the resulting radical sites would be at all the carbons, with some preference for the C-1 and C-6 positions. In many ways, the reactivity of e_s^- , H^\bullet , and OH^\bullet towards saccharides is very much like that towards alcohols and simple ethers. The e_s^- has a low affinity for most of the groups except for the ether linkage and the carbonyl group. The OH^\bullet and H^\bullet , however, will readily abstract a hydrogen, so accessible C–H bonds of lowest energy are most susceptible to abstraction. It is the sequence of reactions available to the glucose ring radicals that affects the final product distribution.

Some of the possible reaction pathways for the C-1 glucose radical are shown in Fig. 7, in which original C–OH bonds are depicted as vertical lines. Abstraction of hydrogen from other molecules with weaker C–H bonds strengths will generate glucose, as will disproportionation, which also leads to the formation of gluconic acids. Other radical transfer reactions followed by abstraction of a hydrogen have been proposed to explain the formation of 2- and 5-deoxygluconic acid. Similar reactions have also been proposed for the C-2 glucose radical. Product identities and *G*-values were established by chromatographic analysis.

Figure 7

Illustrative mechanism for the reactions of the glucose radical formed by loss of hydrogen from the C-1 position; final products are indicated



In the case of glucose ring radicals in oligosaccharides, additional reactions are possible leading to scission of the bond joining the glucose units. Studies of the disaccharide cellobiose show that such scission takes place when the radical sites are at the C-1, C-4 and C-5 positions and that glucose can be formed (52). Accordingly, degradation of large carbohydrates can be initiated, not only by initial reaction at the C-O-C groups, but by abstraction reactions at sites near this linkage.

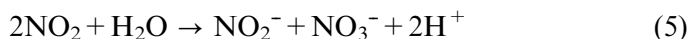
The implication of carbohydrate radiolysis for radiation sterilization of muscle foods is that the chemical consequences will be minor. Except where sucrose is purposely added, the level of carbohydrate in the tissue is small, being about 0.5%. At low concentrations and with relatively low reactivity, carbohydrates are not likely to compete for the primary radicals. Glucose radicals that might be formed could in principle react with the cysteine moiety in albumin to regenerate glucose. These considerations are an extension of those made by Basson et al. in predicting that the radiolysis of sugar in fruits differs substantially from the radiolysis of concentrated sugar solutions (53).

3.4 Minor constituents

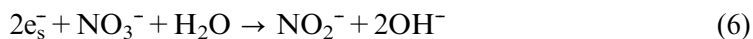
Although the low concentration of even highly reactive minor constituents limits the yield of radiolysis products derived from them, their relevance to food quality, nutrition and safety makes them important.

3.4.1 Salts

Most of the common salts added to foods for diverse reasons, for example, chlorides, sulfates and phosphates, are relatively unreactive towards the primary radicals formed in water. The two exceptions of some significance are nitrates and nitrites, the latter being used for colour preservation in cured meats. Nitrate is highly reactive in solution towards e_s^- and the mechanism of reaction that leads to nitrite is as follows:



The overall stoichiometry is:



which means that one mole of nitrite is formed for every two moles of electrons reacting with a mole of nitrate.

In frozen solutions, the reduction in the yield of e_s^- and the restriction on the mobility of the reacting entities essentially eliminate this reaction (27, 54). It would require extremely high concentrations of nitrate to scavenge electrons.

With respect to radiation sterilization of bacon or ham, the likelihood of reaction (3) is low, not only because the system is frozen, but because the low levels of nitrate or nitrite would have to compete with high levels of constituents that are reactive towards the electron.

3.4.2 Vitamins

Since the sensitivity of vitamins towards irradiation is discussed in the section on nutrition, the radiolysis of vitamin C (ascorbic acid) will be considered here only because the associated chemistry has relevance to other considerations relating to high-dose treatment.

Ascorbic acid, with its carbonyl group and double bond, is highly reactive to e_s^- , H^\bullet and OH^\bullet . It is reduced to an intermediate radical by e_s^- and H^\bullet and is oxidized to the relatively stable tricarbonyl radical ion by OH^\bullet (55, 56). The tricarbonyl radical ion is involved in biochemical processes not initiated by irradiation. Except for a moderate likelihood of reaction with cytochrome-*c* (and presumably with ferrimyoglobin), its most likely reaction pathway is a complex disproportionation reaction

that both regenerates ascorbic acid and produces dehydroascorbic acid, which still has vitamin activity.

3.4.3 **Nucleic acids**

Although nucleic acids represent a very small fraction of the food mass, their radiolysis is of interest, because of its relevance to microbial destruction. The pathways from initial reaction of primary radicals with purine or pyrimidine moieties to ultimate damage to DNA are complex, and the possible interaction with the sugar–phosphate backbone should be considered. For this reason, the reaction with thymine will be illustrated.

As with the other DNA bases, thymine is highly reactive towards e_s^- , H^\bullet and OH^\bullet because of its heterocyclic structure and prevalent carbonyl groups. However, it is the reaction with OH^\bullet that ultimately leads to base damage and in part to single strand breakage in DNA. The OH^\bullet formed either in the hydration sphere (the water bound to DNA) or in the bulk water (surrounding water) reacts by addition, the preferred site being the 5,6-double bond and the unpaired electron residing at either position (57). This radical can also be formed by direct ionization. In either case, the free radical site, at least in single-strand DNA, can transfer to the sugar moiety, which results in scission of the sugar–phosphate link. Direct reaction of OH^\bullet with the sugar in single- or double-strand DNA, which is much less likely, produces the same result. From the standpoint of process efficacy, it is this series of reactions occurring in the nucleus of the contaminating microorganisms that is most important. However, similar reactions with low *G*-values can take place with nucleic acids in muscle cells.

Ward (58), in discussing the implications of such reactions, considers it unlikely that any altered bases in the food could be incorporated into human DNA. Its synthesis involves enzymes that act on precursors of the bases, not on the bases themselves, so competition between normal and altered bases is not a factor. Moreover, if an altered base were somehow incorporated, the DNA polymerases would excise any incorrectly matched base.

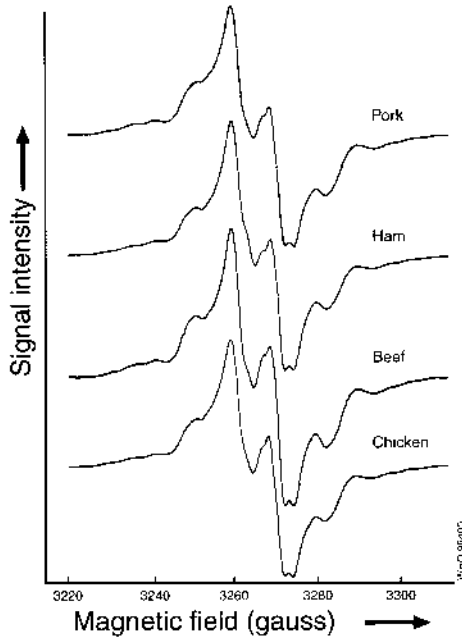
3.5 **Chemical implications: chemiclearance**

This understanding of the radiation chemistry is most important for considering the generic clearance of irradiated foods (29, 59). The operative principles can be stated as follows:

- When foods of similar composition are similarly irradiated, their chemical and microbiological responses are similar and they are, accordingly, toxicologically equivalent.
- When an irradiated food in a class of similar foods is cleared as safe and adequate for consumption, then other members of that class are, correspondingly, wholesome.

Figure 8

Comparison of the electron spin resonance spectra of four different enzyme-inactivated muscle foods irradiated to 50 kGy at -40°C ^a



^a Reproduced from Taub (25) with the permission of the publisher.

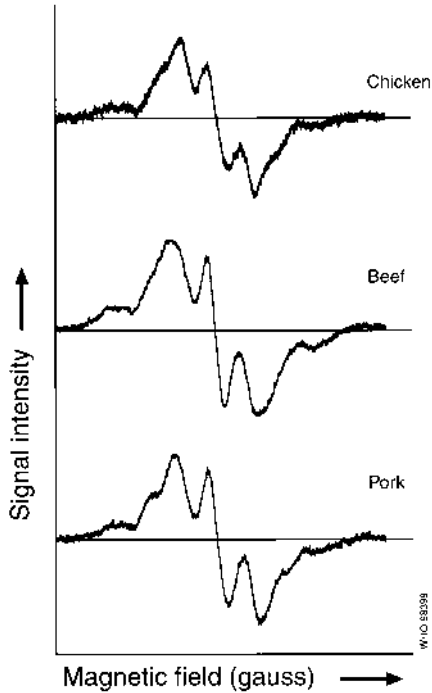
Applying these principles to high-dose irradiated precooked moist muscle foods involves: demonstrating the commonality and similarity in chemical responses among these and other foods expected to be so processed; determining that the microbiological, nutritional and toxicological data on tested foods confirm the wholesomeness of those foods; and, by reference to the tested foods, extrapolating the determination of their safety to the other foods, so the generic class of irradiated foods is “chemicleared.” The data acquired over the years showing this commonality in the nature and behaviour of the intermediate radicals and in the yield of stable radiolysis products resulting from the involvement of these and other entities are described below. A diverse set of foods irradiated over a wide range of dose has been examined using ESR techniques to detect radicals and chromatographic analysis to quantify the yields of products.

3.5.1 *Commonality of intermediates*

Irrespective of the nature and condition of the muscle foods, the same type and behaviour of protein-derived and lipid-derived radicals are observed in all of them upon irradiation (59). The most striking illustration of this commonality is shown in Fig. 8 in which the ESR spectra of enzyme-inactivated pork, ham, beef and chicken irradiated to

Figure 9

Comparison of the electron spin resonance spectra of fats from chicken, beef and pork irradiated to 50 kGy at -40°C ^a

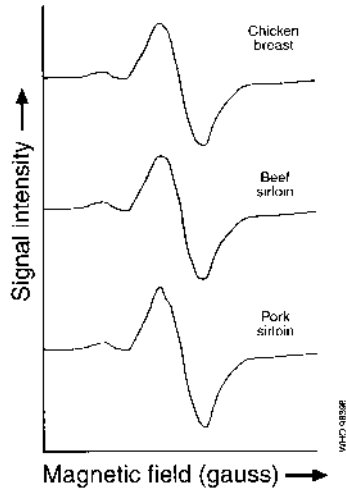


^a Reproduced from Taub et al. (29) with the permission of the publisher.

50 kGy at -40°C are compared. They are essentially all the same, and reflect the contribution of the myosin and lipid radicals; the latter would differ slightly among them owing to different fatty acid compositions. This common pattern indicates that the processes by which the radicals stable at this temperature are formed are all similar. The subsequent behaviour of the protein radicals upon thawing, leading to some aggregation and degradation, must also be similar, since the gel electrophoretic patterns of the extracted proteins are all similar. To examine more closely the commonality in the triglyceride radicals, the fats from these meats were separately irradiated and their spectra at -40°C were compared, as shown in Fig. 9 for chicken, beef and pork fats. Here too the spectra are similar, the small differences among them being attributable to differences in the triglycerides in these meats.

More recent data on cooked and uncooked foods irradiated to lower doses and monitored at different temperatures further illustrate the commonality of effects (60). Raw chicken breast, beef sirloin and pork sirloin were irradiated to 1 kGy at 77 K in order to compare their spectra and the yields of radicals responsible for such spectra. In all cases, as

Figure 10
Comparison of the electron spin resonance spectra of three uncooked muscle foods irradiated to 1 kGy at 77 K



expected, the dominant feature in the spectra is a broad singlet (Fig. 10) whose intensity was the same for all meats and increased with dose (Fig. 11). Warming to -78°C produces the expected dominant asymmetric doublet and, upon further amplification, the contributions of the addition radicals in the low and high field regions. Comparison of raw turkey breast with roasted/precooked turkey, irradiated to 3.8 kGy and examined at -78°C , shows no perceptible difference, indicating that protein denaturation does not affect the formation of the protein radicals. However, comparison of the radical stability as a function of temperature

Figure 11
Comparison of the yield-dose relation of radicals from three uncooked muscle foods irradiated at 77 K, based on the electron spin resonance signal

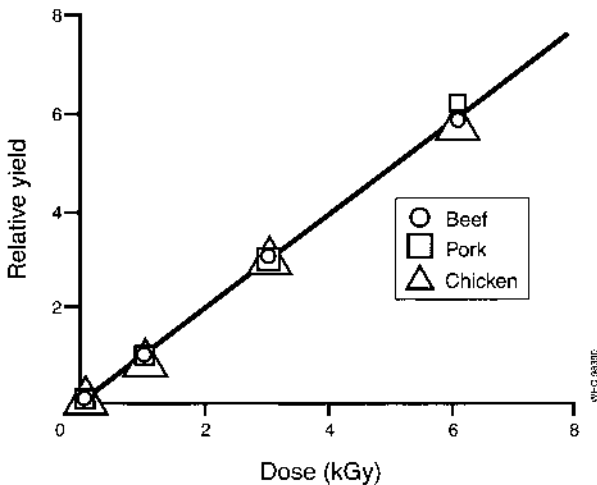
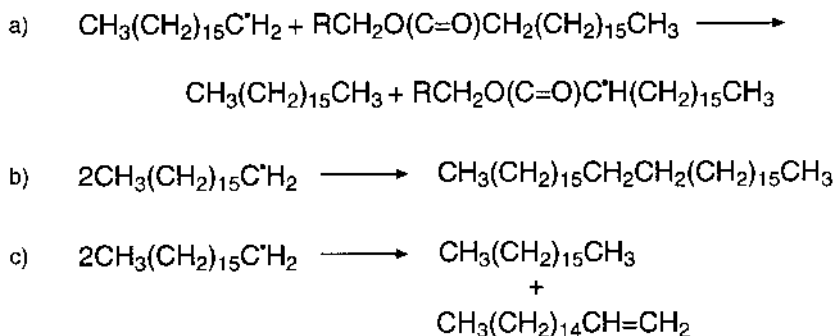


Figure 12

Potential pathways for reaction of the alkyl (C₁₇) radical (from stearic acid): abstraction; combination to form the dimer; and disproportionation to form a double bond at a terminal carbon



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suggests that some difference in the structure of the meat at about -60°C could affect radical mobility and reactivity, since the radicals decay more rapidly in chicken than in beef or pork. This matrix effect is more clearly discerned in the case of the lipid radicals; those in chicken fat decay much more rapidly. Chicken fat has more linoleic acid and is less viscous, which is consistent with higher mobility and reactivity.

3.5.2 Commonality of lipid-derived volatile products

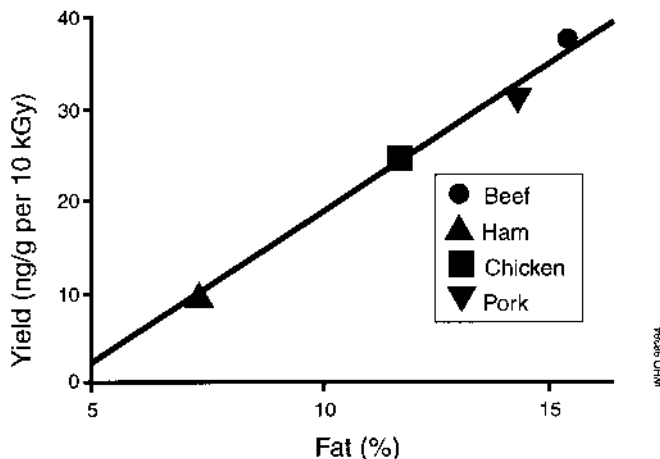
Although the likelihood of C–C or C–O bond scission in the fatty acid moieties bound to the glycerol structure in a triglyceride is significantly smaller than that of other processes described above, the sensitivity of chromatographic analyses makes it possible to detect the resultant volatile hydrocarbon products and to relate them to their precursor fatty acids (29, 59, 61; K. M. Morehouse, unpublished data). Such scission processes lead to different alkyl, acyl and acyloxy radicals. The alkyl radicals can form stable components by abstraction, by combination to form dimeric compounds, and by disproportionation to form two hydrocarbons, one with a double bond at the terminal end (Fig. 12). It is from these types of radical reactions that evidence for commonality and predictability can be obtained.

Dependence on total fat

In the case of C–C bond scission in the fatty acid chain, the resultant products with about six carbons or less will be the same irrespective of the particular fatty acid. Consequently, the yield of pentane, hexane and even heptane and octane should be closely related to the total amount of fat in the sample. This prediction is confirmed by comparing as a function of fat content the yields of such hydrocarbons from enzyme-inactivated ham, chicken, pork and beef irradiated over a range of dose (29).

Figure 13

Normalized yield of hexane as a function of fat content in irradiated enzyme-inactivated muscle foods, expressed as nanograms per gram and normalized to 10 kGy of dose applied at $-30^{\circ}\text{C}^{\text{a}}$



^a Reproduced from Taub et al. (29) with the permission of the publisher.

Fig. 13 shows that the yield of hexane, normalized in terms of ng/g per 10 kGy of dose, is linearly dependent on the proportion of fat in these products, which ranges from 7.3% for the ham to 15.4% for the beef. Similar results were obtained for other hydrocarbons.

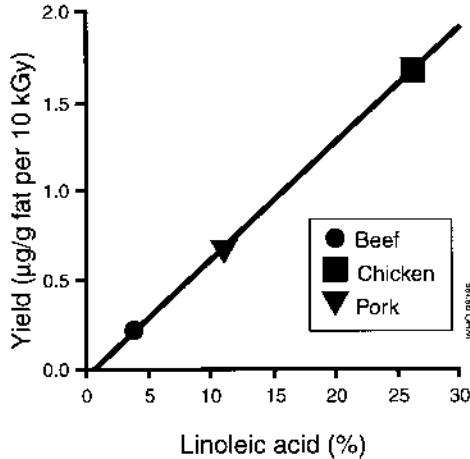
Dependence on fatty acids

Since C–C bond scission at the alpha- and beta-positions relative to the carbonyl group in the fatty acid moieties can occur, the subsequent abstraction reaction will result in hydrocarbons with one or two fewer carbon atoms (C_{n-1} and C_{n-2}), respectively. Similarly, the alternative disproportionation reaction will result in C_{n-1} and C_{n-2} hydrocarbons with an added double bond. It can thus be predicted that the yield of certain volatile hydrocarbons will depend on the level of the precursor fatty acid in the triglycerides of the foods being irradiated. This prediction is confirmed in the analysis of C_{14} to C_{17} hydrocarbons formed in different irradiated muscle food products (29, 61; K. M. Morehouse, unpublished data).

The yield of heptadecadiene ($C_{17:2}$) is particularly instructive, because the level of precursor linoleic acid differs substantially among the chicken, pork and beef products described above, the level in chicken being about six times that in beef. As Fig. 14 illustrates, the yield of $C_{17:2}$ normalized per gram of fat per 10 kGy of dose is linearly dependent on the proportion of linoleic acid in the fat. This relationship also holds for uncooked products irradiated in the chilled state over a lower dose range.

Figure 14

Normalized yield of heptadecadiene ($C_{17:2}$) as a function of linoleic acid content in irradiated, enzyme-inactivated muscle foods, expressed as micrograms of the hydrocarbon per gram of fat and normalized to 10 kGy of dose applied at $-30^{\circ}C^a$

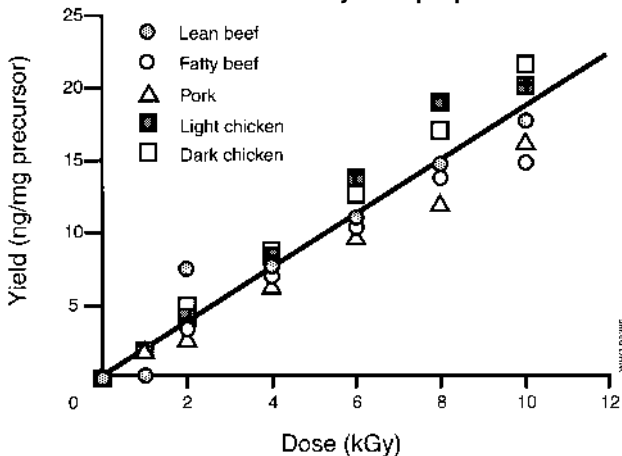


^a Reproduced from Merritt et al. (61) with the permission of the publisher.

The yield of hexadecatriene ($C_{16:3}$) is also instructive, because it is formed in part when the C_{n-2} radical from linoleic acid reacts by disproportionation and acquires a terminal double bond. Analyses of $C_{16:3}$ from five different uncooked products irradiated in the chilled state show that the normalized yields are linearly dependent on dose and have essentially the same slope (Fig. 15) (K. M. Morehouse, unpublished data). Such similarity in the G -values implies that the formation of the radical and

Figure 15

Normalized yield of hexadecatriene ($C_{16:3}$) as a function of dose in irradiated raw muscle foods, expressed as nanograms of the hydrocarbon per milligram of precursor fatty acid which in this case is linoleic acid; the hydrocarbon is formed by scission at the beta carbon followed by a disproportionation reaction



its subsequent reactions are essentially independent of the molecular environment in which the precursor fatty acid moiety exists.

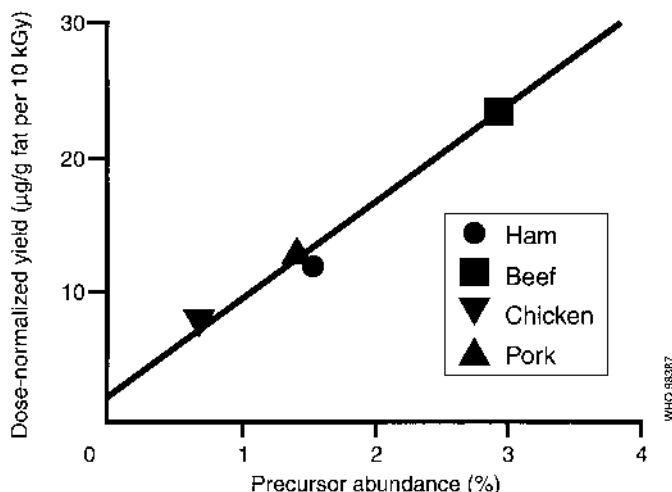
Dependence on triglycerides

Since the major fate of electrons formed in the ionization process is to react by dissociative attachment to the carbonyl group in any fatty acid moiety of the constituent triglycerides, an equal number of stable fatty acid anions and propanedioldiester radicals will be formed. To emphasize the positional differences of the fatty acid moieties, these radicals will be denoted here as $\text{H}_2\text{C}(\text{O}_2\text{R}'')\text{CH}(\text{O}_2\text{R}')\dot{\text{C}}\text{H}_2$, showing the loss of $\cdot\text{O}_2\text{R}$ from the 1-position. Upon abstracting a hydrogen from other triglycerides, they become stable propanedioldiester products, $\text{H}_2\text{C}(\text{O}_2\text{R}'')\text{CH}(\text{O}_2\text{R}')\text{CH}_3$. Accordingly, if scission is equally likely from the 1, 2, or 3 position in the glycerol backbone, then the yield of corresponding dioldiester isomers, namely $\text{H}_2\text{C}(\text{O}_2\text{R}'')\text{CH}(\text{O}_2\text{R}')\text{CH}_3$, $\text{H}_2\text{C}(\text{O}_2\text{R}'')\text{CH}_2\text{CH}_2(\text{O}_2\text{R})$, or $\text{H}_3\text{CCH}(\text{O}_2\text{R}'')\text{CH}_2(\text{O}_2\text{R})$, will correlate with the level of precursor triglycerides containing the relevant $\text{O}_2\text{R}''$, $\text{O}_2\text{R}'$, and O_2R fatty acids.

This correlation has been shown in a limited number of cases examined (29). Non-volatile propanedioldiesters were isolated from enzyme-inactivated chicken, beef, pork and ham samples irradiated to 30, 60 and 90 kGy. Propanedioldipalmitate and propanediolpalmitateoleate yields were found to increase linearly with dose, the slopes being different in each product. Since analyses of a specific triglyceride could not be made at the time of this study, their abundances in each product were estimated on the basis of the fatty acid composition and certain assumptions about their biosynthetic combinations. For example, the relative abundance of the two triglycerides that in one product would contain two palmitate (P) moieties and could be precursors of propanedioldipalmitate was 7.1% for POP and 5.3% for PPL, where O and L denote oleate and linoleate, respectively. In each case there is a 1 in 3 chance that scission would eliminate the O or L moiety and form the dipalmitate. As Fig. 16 shows, a plot of the normalized yield of propanedioldipalmitate (i.e. the slope of the yield-dose plot) against the percentage abundance of precursor triglycerides in the chicken, pork, ham and beef samples conforms extremely well to a linear dependence (61). This again implies that the formation and reaction of the relevant radicals is not especially sensitive to the specific molecular environment of the precursor triglycerides. It also further confirms the commonality in the chemistry among diverse triglycerides and the prediction of shifts in product distribution based on a knowledge of compositional differences.

Figure 16

Relationship of the dose-normalized yield^a of propanedioldipalmitate to its triglyceride precursors in four enzyme-inactivated muscle foods irradiated to 30, 60, and 90 kGy at -40 °C^b



^a This normalized yield is derived from the slope of a yield-dose plot for each food, which is proportional to *G*-value, and is expressed on the ordinate in terms of micrograms of the propanedioldipalmitate per gram of fat per 10 kGy. The abundance of the precursor triglycerides (see section 3.5.2) is expressed on the abscissa in terms of percentage of total triglycerides.

^b Reproduced from Merritt et al. (61) with the permission of the publisher.

3.5.3 General implications

Although detailed studies of the type described above, in which normalized yields of radiolysis products are compared for different foods, have not been conducted systematically, there is sufficient evidence to substantiate the principles stated here and to apply them to different food classes. The many studies on proteins in diverse foods irradiated to low and high doses are consistent with the chemistry described. Studies on volatile and non-volatile products derived from fatty acids, fatty acid esters and oils, in addition to those described above, also show a consistency in the chemistry (62). The use of volatile hydrocarbon analyses to detect and confirm that diverse lipid-containing foods have been irradiated also attests to this consistency. Results similar to those for proteins and lipids have also been obtained with diverse starches and glucose oligomers (47, 48). They show that the radicals formed in cereals are the same as those formed in pure starches: all have the same ESR spectral characteristics and the same decay dependence on water content and storage time (63). Consequently, the commonality of effects in starch-derived products has also been demonstrated. The very careful measurements in diverse animal sources of almost identical thiamine retention, except for ostrich, further

emphasize the consistent chemistry (64). It follows then that variations in the food matrix containing these same constituents would not alter significantly the course of reactions described here and, consequently, would not affect safety.

It is important to stress, however, that in the studies discussed relating to the high-dose irradiation of meats, the irradiation was always carried out on samples in the frozen state and in the absence of oxygen. Freezing reduces the yield of primary entities and related products, particularly in the aqueous phase, the level of chemical processes being reduced to 20% of that occurring in the nonfrozen aqueous phase. The elimination of oxygen significantly reduces the formation of certain undesirable oxidation products (from lipids) and avoids the loss of certain flavour compounds (in spices). Any residual oxygen in the muscle matrix is radiolytically reduced by the first 0.6 kGy of dose. Accordingly, food constituents in raw or processed foods irradiated in the chilled state or at ambient temperature in the presence of oxygen to a dose of 10 kGy would be chemically affected to an extent generally comparable to that seen in precooked, vacuum-packed foods irradiated while frozen to a dose of 50 kGy.

By using the principles of commonality and predictability and by relying on studies that have been summarized here and elsewhere, it is possible to extrapolate the results from the radiolysis of model compounds and meats to other food commodities in order to assess the chemical changes that would occur when these foods are irradiated, separately or together with meats, at absorbed doses *above* 10 kGy. On the basis of the commonality in the radiation chemistry of different proteins, lipids and starches, it can be concluded that the irradiation of food commodities other than meats will lead to the spectrum of radiolysis products previously determined for the irradiation of related food constituents to doses *below* 10 kGy. Furthermore, increasing the absorbed dose will lead to an increase in the level of the radiolytically-generated products, but not necessarily to a change in the spectrum of products. Therefore, irradiation of other foods (e.g. potatoes, tomatoes, vegetables or spices) to high doses, alone or together as part of frozen meals or as an ingredient with the meat, will not lead to the formation of chemical entities that have not been previously identified. For these reasons, comparable food products that might be formulated differently from those described here – structured in different ways by comminution, combined with still other food commodities, or subjected to combined processing techniques – would still reflect similar chemical consequences and should not need to be separately tested for wholesomeness. It would suffice, where necessary, to provide data on the consistency in the chemistry.

3.6 Conclusions

The knowledge of what can and does occur chemically in high-dose irradiated foods, which derives from over 70 years of research on radiation chemistry and from over 50 years of research on the radiolysis of food, justifies the following conclusions:

- Reactions initiated by the irradiation process follow pathways for each major constituent that are predictable and that depend on processing conditions.
- Overall chemical change, as reflected either in the formation of a stable compound or the loss of a particular constituent, is quantifiable and relatively minor, requiring sensitive techniques to discern that a product had been irradiated.
- Yields of any product derived from a major constituent will depend linearly on dose, but yields from a minor constituent could remain constant or even decrease once the dose corresponding to the depletion of that constituent is reached.
- As a consequence of the penetrating power of the radiation permitted for use and of the associated energy deposition process, the yield of products formed or lost throughout the irradiated food will be relatively uniform, varying by less than about $\pm 25\%$.
- As a consequence primarily of the effect of phase, irradiating moist foods while frozen and in the absence of oxygen significantly decreases the overall chemical yields by about 80%, so the cumulative effects of irradiating to a dose of 50 kGy at $-30\text{ }^{\circ}\text{C}$ is essentially equivalent to a dose of 10 kGy at room or chilled temperatures.
- Compounds found in irradiated model systems that are either far different in composition from the foods of interest or have been irradiated under extreme conditions do not validly reflect the chemistry (or toxicology) of actual foods, because competitive reactions will occur in the latter that make the formation of such compounds very unlikely.
- Virtually all of the radiolysis products found in high-dose irradiated foods to date are either naturally present in foods or produced in thermally processed foods, a radiolysis product being defined as a compound that originates from a food constituent during irradiation and that, at least initially, increases in yield with increasing dose.
- This understanding of the radiation chemistry of foods is vital in assessing wholesomeness.
- The commonality in the chemistry among the major protein, lipid and starch constituents, with minor chemical differences being accounted for by the slight differences in the composition of these constituents, justifies use of the chemiclearance approach for granting broadly-based, generic approvals of high-dose irradiated foods.

4. Nutritional considerations

4.1 Commonality and predictability

Numerous investigations have been carried out to study the nutritional adequacy of irradiated foods under various conditions, many of which have considered the effects of high-dose irradiation. Several reviews of this work summarize the results obtained (65–71).

In general, these investigations have confirmed the principles of commonality and predictability of radiation effects discussed in section 3. Loss of nutrients increases with radiation dose, but the rate of loss can differ substantially. Some nutrients are very stable to irradiation and show no important losses, even at the high doses considered here, while others are more affected. Factors modifying the effects of radiation, such as oxygen, water or temperature, will affect different foods to about the same extent. For example, thiamine and the tocopherols are radiation-sensitive in any food, whereas riboflavin is much more stable, as confirmed by recent studies in pork, beef, lamb and turkey (72, 73), mackerel (74) and prawns (75).

Certain patterns of radiation response are observed in all foods and are therefore recognized as common and predictable. However, the complexities of the radiation chemistry in different foods are not understood in every detail as the following observations illustrate. Radiation-induced loss of α -tocopherol was found to be consistently greater in turkey breast than in beef, pork, lamb or turkey leg (72); loss of thiamine was somewhat greater in beef and turkey breast than in lamb, pork and turkey leg (73). Analyses of sulfhydryl, protein, moisture, fat or water content, pH, or reducing capacity by redox titration provided no explanation for these differences in retention. However, there is a possibility that certain constituents can react with intermediate vitamin radicals and regenerate the original vitamin, as is the case with α -tocopherol radicals and ascorbate; such “sacrificial” loss of the reactive constituent could lower the vitamin loss and affect its apparent radiation sensitivity.

4.2 Macronutrients

Animal feeding studies have shown that foods treated with the radiation doses considered in this report are not adversely affected with regard to the metabolizable energy of their carbohydrates, lipids and proteins. An irradiation dose of 56 kGy had no effect on the biological availability of the macronutrients in nine food items (76). Balance studies in human volunteers consuming a variety of foods irradiated with a dose of 28 kGy revealed no effects of irradiation on metabolizable energy, nitrogen balance or coefficients of digestibility (77).

While fats and carbohydrates in food serve primarily as sources of energy, proteins provide essential amino acids, which the human organism needs to make its own proteins. Particular attention has therefore been paid to the possible effects of radiation on the biological value and digestibility of food proteins. A comprehensive toxicological investigation of chicken meat radiation-sterilized with a dose of 59 kGy by electron beam or gamma-rays involved the determination of the protein efficiency ratio (PER) of the chicken meat by rat growth assay; no effect of irradiation was observed (9). The amino acid pattern of the irradiated chicken meat was also unaffected (78).

Results obtained on mackerel irradiated to doses of up to 45 kGy are presented in Table 1 (79). Protein quality, here expressed as net protein utilization (NPU), was not adversely affected by irradiation, as evident from the absence of any trend with dose. The same authors also determined the amino acid composition of mackerel proteins by chemical analysis in samples irradiated to average doses of up to 45 kGy and found no significant effects of irradiation. The rat growth assay showed no effect at this dose level on the protein quality of cod, whereas amino acid analysis indicated some loss of cysteine/cystine (80). Curiously, the cystine levels in the irradiated samples showed no dependence on dose, suggesting that the analysis of the non-irradiated control samples may have been in error.

With regard to foods of plant origin, a dose of 28 kGy had no effect on the biological value of corn protein or wheat gluten (81). Irradiation of cereals with high doses was repeatedly found to improve somewhat the nutritional value of cereal proteins as determined in chick growth assays (82, 83). For example, wheat bran irradiated to 50 kGy had an NPU of 40.3%, which was significantly higher than that of non-irradiated bran which was 36.0% (82).

Table 1
Evaluation of the nutritional value of proteins in gamma-irradiated mackerel by the rat growth assay

Radiation dose (kGy)	True digestibility (%)	Biological value (%)	Net protein utilization (%)
0	93.2	82.6	77.0
1	94.8	84.2	79.8
3	96.6	84.8	81.9
6	97.0	85.9	83.3
10	98.1	84.1	82.6
25	97.0	82.6	80.1
45	98.6	80.2	79.1

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Table 2

The effect of irradiation on the protein quality of a rat diet

Radiation dose (kGy)	True digestibility (%)	Biological value (%)	Net protein utilization (%)
0	85.6	80.5	68.9
5	83.6	75.8	63.5
10	86.5	81.7	70.6
25	87.0	78.1	68.0
30	84.8	77.3	65.4
70	85.3	76.4	65.2

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The good growth observed in various animal species fed different kinds of irradiated feeds supports the conclusion that digestibility and biological value of proteins are not adversely affected by treatment with radiation doses of up to 70 kGy (84). Results obtained with a rat diet are shown in Table 2 (85).

Legume seeds irradiated with very high doses (210 kGy for field beans, 180 kGy for lentils) were found to have an improved protein nutritional value when tested in chicks (86, 87). The evidence from animal feeding studies and from chemical analyses indicates that nutritionally relevant losses of protein quality do not occur in the dose range up to about 70 kGy. The absence of any significant trend with dose suggests that even higher doses would not be of concern.

4.3 Vitamins

Like thermal treatments, radiation processing of foods causes some loss of vitamins. Work summarized in the reviews mentioned in section 4.1 (65–71) has shown that some vitamins are quite insensitive to ionizing radiation, whereas others are rather radiation sensitive. Table 3 gives an overview (88). However, this ranking of sensitivities is not always strictly applicable. Many factors influence the radiation resistance of a vitamin, such as the composition of the food under consideration, the packaging atmosphere, and the temperature during irradiation and post-irradiation storage. The presence or absence of oxygen in the packaging atmosphere has a particularly pronounced effect in the case of vitamin E. In beef irradiated to 30 kGy under nitrogen, no loss of vitamin E was found; however, when the meat was irradiated in the presence of air, a loss of 37% was observed (87). Irradiation of chick feed with a dose of 50 kGy resulted in a 10% loss of vitamin E when the feed was vacuum packed, but a loss of 51% when it was packed in air (90).

Although vitamin losses generally increase with increasing radiation dose, irradiation of foods with high doses often requires processing

Table 3

Relative radiation sensitivity of vitamins

Most sensitive	—————▶	least sensitive
Fat-soluble vitamins		
Vit. E → Carotene → Vit. A → Vit. D → Vit. K		
Water-soluble vitamins		
Vit. B ₁ (thiamine) → Vit. C → Vit. B ₆ → Vit. B ₂ → Folate, nicotinic acid (niacin) → Vit. B ₁₂		

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conditions that minimize undesirable sensory effects, conditions that also contribute to a reduction in vitamin losses. Oxygen must be excluded, e.g. by vacuum packaging, and the irradiation is usually carried out at cryogenic temperatures. Under these conditions, even vitamins generally considered as radiation sensitive may be well protected. For example, the radiation-sterilized chicken meat used for the Raltech feeding studies (see section 6) was processed in the following way: enzyme inactivation (blanching) by heating to an internal temperature of 73–80 °C, vacuum packaging, and irradiation at –25 °C with either gamma-rays or 10 MeV electrons. Control samples were kept frozen and another series was thermally processed at 115.6 °C (9).

As can be seen from Table 4, neither electron nor gamma-ray irradiation had a significant adverse effect on sample content of vitamin B₁₂, riboflavin, pyridoxine, nicotinic acid (niacin), pantothenic acid, biotin, folic acid or vitamins A, D and K, when compared to the frozen control (78). The gamma-ray-irradiated lot had 32% less and the thermally sterilized lot 34% less thiamine than the frozen control; the electron-irradiated lot had 14% less thiamine than the frozen control, but the difference was not considered statistically significant. Thus, with the sole exception of thiamine in the gamma-ray-irradiated lot, none of the vitamins investigated was significantly diminished by irradiation, in spite of the high average radiation dose of 59 kGy.

The processing conditions for radiation-sterilized chicken meat described in the previous paragraph are essentially those developed at the Natick Laboratories of the United States Army for sterilizing various kinds of meat and meat products. Under those conditions, thiamine retention in radiation-sterilized pork was better than in heat-sterilized pork (91).

Data from the study relating to the effect of subfreezing temperatures and of radiation source are presented in Fig. 17. The data demonstrate once again the improved retention of thiamine when irradiation is carried out at lower temperatures. The data also demonstrate the much

Table 4
**Vitamin content of frozen, thermally processed, gamma-irradiated
 and electron-irradiated enzyme-inactivated chicken meat^a**

Vitamin	Process			
	Frozen control	Heat-sterilized	Gamma-irradiated (59 kGy at -25 °C)	Electron-irradiated (59 kGy at -25 °C)
Thiamine hydrochloride (mg/kg)	2.31	1.53 ^b	1.57 ^b	1.98
Riboflavin (mg/kg)	4.32	4.60	4.46	4.90 ^c
Pyridoxine (mg/kg)	7.26	7.62	5.32	6.70
Nicotinic acid (niacin) (mg/kg)	212.9	213.9	197.9	208.2
Pantothenic acid (mg/kg)	24.0	21.8	23.5	24.9
Biotin (mg/kg)	0.093	0.097	0.098	0.103
Folic acid (mg/kg)	0.83	1.22	1.26	1.47 ^c
Vitamin A (IU/kg)	2716	2340	2270	2270
Vitamin D (IU/kg)	375.1	342.8	354.0	466.1
Vitamin K (mg/kg)	1.29	1.01	0.81	0.85
Vitamin B ₁₂ (mg/kg)	0.008	0.016 ^c	0.014 ^c	0.009

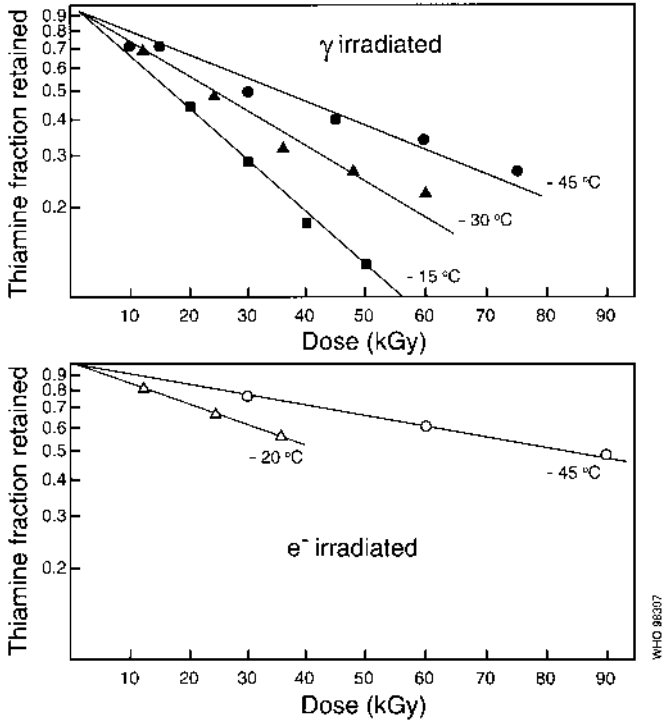
^a Vitamin concentrations are given on a dry weight basis.

^b Significantly lower than frozen control.

^c Significantly higher than frozen control.

Adapted from Thayer (78) with the permission of the publisher.

Figure 17
The effect of radiation dose and temperature during irradiation on thiamine retention in pork^a



^a Reproduced from Thomas et al. (91) with the permission of the publisher. The initial concentration of thiamine was 0.9 mg/100 g (from reference 91)

better retention of thiamine with electron irradiation than with gamma-ray irradiation (an effect also seen in the case of chicken meat, Table 4). The authors ascribe this higher retention to the much higher dose rate delivered by the electron beam, which favours radical-radical reactions over radical-substrate reactions (91).

Thiamine (vitamin B₁) is the most radiation-sensitive of the water-soluble vitamins. Using chemical, microbiological and rat growth assay methods, 60–70% of thiamine in beef was found to be destroyed by a dose of 30 kGy delivered under less than ideal conditions (samples were sealed in cans, but apparently oxygen was not excluded before sealing; moreover, samples were shipped frozen to the irradiation facility, but the temperature during irradiation was not indicated) (92).

The protective effect of irradiating at low temperatures was first recognized in studies carried out in the United States and reported in 1947 (93). Subsequent investigations in the United Kingdom have shown

Table 5
Thiamine in raw, fried, irradiated-fried, and fried-irradiated bacon at three radiation doses and two irradiation temperatures

Treatment	Radiation dose (kGy)	Thiamine (mg/100 g protein)	Irradiation temperature 2 °C			Irradiation temperature -40 °C		
			Due to irradiation	Due to combined treatment	Due to frying	Due to irradiation	Due to combined treatment	Due to frying
None (raw)	0	4.42	—	—	—	—	—	—
	7.5	1.77	60 ^a	—	—	15 ^a	—	—
	15.0	0.95	78 ^a	—	—	32 ^a	—	—
	30.0	0.40	91 ^a	—	—	62 ^a	—	—
Irradiated, then fried	0	2.28	—	48 ^a	48 ^b	—	50 ^a	50 ^b
	7.5	0.76	—	83 ^a	57 ^b	—	62 ^a	55 ^b
	15.0	0.40	—	91 ^a	58 ^b	—	70 ^a	57 ^b
	30.0	0.07	—	98 ^a	82 ^b	—	96 ^a	91 ^b
Fried, then irradiated	0	2.32	—	47 ^a	47 ^b	—	52 ^a	52 ^b
	7.5	2.02	13 ^c	54 ^a	—	11 ^c	57 ^a	—
	15.0	1.78	23 ^c	60 ^a	—	21 ^c	62 ^a	—
	30.0	1.49	36 ^c	66 ^a	—	32 ^c	67 ^a	—

^a Compared to non-irradiated, non-fried samples.

^b Compared to non-fried samples irradiated to the same dose.

^c Compared to non-irradiated, fried samples.

Adapted from Thayer et al. (96) with the permission of the publisher.

that this benefit also applies to the retention of thiamine: when minced beef at different temperatures was electron-irradiated to a dose of 10kGy, the loss of thiamine was 65% at room temperature, 24% at -10°C , 12% at -20°C , and 5% at -75°C (samples sealed in cans under nitrogen) (94). To provide microbiologically safe diet items to immunosuppressed patients, dairy products were packaged under nitrogen and irradiated to a dose of 40 kGy at -78°C ; yoghurt bars and nonfat dry milk lost about 25% of their thiamine content whereas in ice cream, mozzarella cheese and cheddar cheese, thiamine levels were unaffected by irradiation (95).

The combined effect of irradiation and frying on thiamine in bacon was more than a simple addition if the bacon was first irradiated and then fried, as shown in Table 5 (samples were vacuum packaged in barrier pouches and irradiated at 2°C or -40°C). In the dose range up to 15 kGy the synergistic effect was small; at 30 kGy it appeared to be substantial. In contrast, when bacon was first fried and then irradiated, the combined effect on thiamine was smaller than expected on the basis of adding the effects of irradiation and heating together, possibly as a result of the lower water content of fried bacon (96).

Because an earlier study had suggested that irradiation might have caused the formation of antimetabolites to thiamine and pyridoxine in meats, a study of the possible occurrence of antithiamine and antipyridoxine factors in irradiated chicken and beef was carried out (97). No evidence of antivitamin factors was found in any of the meat tested.

Irradiation of ground beef (samples sealed in cans, apparently without the exclusion of air, and transported frozen to the irradiation facility; temperature during irradiation not indicated) to a dose of 30 kGy caused losses of 68% thiamine, 25% pyridoxine and 8% riboflavin indicating the relatively high radiation sensitivity of thiamine, low sensitivity of riboflavin, and intermediate position of pyridoxine (98). When six foodstuffs (beef liver, chicken, cabbage, green beans, lima beans and sweet potatoes) were irradiated to doses of 28 and 56 kGy, the observed losses of pyridoxine ranged from 0% and 18% in beef liver to 48% and 76% in sweet potatoes (99). Irradiation of pork to 30 kGy caused no loss of pyridoxine when assayed in the raw or cooked state (100).

No significant loss of riboflavin was noted in cheddar and mozzarella cheeses, yoghurt bars, ice cream, and nonfat dry milk sterilized with a dose of 40 kGy at -78°C in a nitrogen atmosphere (95).

Vitamin B₁₂ is quite insensitive to irradiation. No loss was observed in haddock fillets irradiated to 25 kGy (101), in various kinds of fish irradiated to a dose of 30 kGy (102) or in dairy products sterilized with a

dose of 40 kGy at -78°C in a nitrogen atmosphere (95). The data presented in Table 4 indicate no loss of this vitamin in radiation-sterilized chicken meat (78).

Many studies attest to the low radiation sensitivity of niacin. No loss of this vitamin was observed in ground beef irradiated to a dose of 30 kGy (99) (see also Table 4).

No loss of folic acid was found in radiation-sterilized beef (103). A chick diet irradiated to a dose of 28 kGy also possessed full folic acid activity (104). In view of the limited number of reports available at that time on folic acid in irradiated food, the Joint FAO/IAEA/WHO Expert Committee on the Wholesomeness of Irradiated Food, at its meeting in 1980, recommended additional studies (1). No loss of folic acid was observed in radiation-sterilized chicken meat (average dose 59 kGy) as indicated in Table 4 (78). A relatively low radiation sensitivity of folic acid and of the many folate vitamers was confirmed by studies on effects of irradiation on folate levels in several foodstuffs (105) and on bioavailability of folates (106). However, in these studies the radiation dose applied did not exceed 10 kGy. Vegetables, the main dietary source of folates, are anyway unlikely candidates for high-dose irradiation.

Vitamin C is a radiation-sensitive vitamin. The most important sources of vitamin C in human nutrition are fresh fruits and fruit juices, vegetables and potatoes. Experience has shown that these products are generally unsuitable for high-dose irradiation because such treatment would cause undesirable changes in their sensory qualities. No loss of vitamin C was observed in onion powder even when the extremely high dose of 270 kGy was applied to samples sealed in tin cans, or when a 20-kGy dose was applied to samples irradiated in commercial 22.5-kg (50-lb) boxes (107). The ascorbic acid level in ground paprika was reported to be unaffected even by a sterilizing dose, but no experimental data were presented in this review paper (108).

The vitamin A content of fillets of dogfish irradiated at 0°C to 3 kGy was unaffected; 45% was lost after treatment with 30 kGy (102). Since radiation-induced losses depend on the temperature and the atmosphere during irradiation, the results for vitamin A in cream cheese are especially instructive. Determinations made four weeks after the irradiation of cream cheese to 50 kGy indicated that 5% of the vitamin A was lost when irradiation was undertaken under vacuum at ambient temperature, 5% with irradiation in air at -80°C , and 60% with irradiation in air at ambient temperature (109).

Most of the foods that are important sources of vitamin A in the human diet, such as milk, butter and cheese, are not among the products consid-

ered for commercial high-dose irradiation. Carotene (provitamin A), like vitamin C, is primarily provided by vegetables and fruits that are likewise unsuitable for high-dose processing.

Vitamin D is less radiation-sensitive than vitamin A (110). No loss of vitamin D was observed in radiation-sterilized chicken meat prepared for the Raltech feeding studies (see Table 4).

In spite of the sensitivity of vitamin E to irradiation, consumption of irradiated food cannot be expected to lead to an insufficient supply in humans, because the main sources of vitamin E in human nutrition are margarine, butter and vegetable fats, and oils. None of these foodstuffs presents a microbiological problem, and there would be no reason to irradiate them. In addition, most high-fat foods suffer undesirable changes in sensory quality when irradiated to high doses.

Early studies established vitamin K as the least radiation-sensitive of the fat-soluble vitamins (110). Its insensitivity was especially evident in vegetables: broccoli, cabbage, spinach and some other vegetables irradiated to 28 or 56 kGy and stored for 9 or 15 months at room temperature showed no loss of vitamin K activity (111). However, vitamin K appears to be less stable in beef, where the levels are very low. After irradiation with 28 or 56 kGy, a rat diet, comprising 35% beef, that had a barely sufficient level of vitamin K caused severe vitamin K-deficiency (haemorrhagic syndrome) in male rats (112). This deficiency caused considerable concern at the time, because it was thought that irradiation had produced an anticoagulant factor in beef. Continued investigations established that this was not the case (113). The loss in the irradiated chicken meat prepared for the Raltech feeding study was about 36%, but was not statistically significant (Table 4), presumably because the vitamin K levels were close to the detection limit of the analytical method.

Taken together, these studies indicate that, except for thiamine, the loss of vitamins following high-dose irradiation of foods is insignificant and not a concern. For thiamine, the impact on dietary intake needs to be considered.

4.4. Polyunsaturated fatty acids

Certain polyunsaturated fatty acids (PUFAs) are essential in human nutrition. Consequently, the reported destruction of highly unsaturated fatty acids in herring oil with doses of 2 or 10 kGy (114) caused concern about the stability of PUFAs in irradiated, fat-containing foodstuffs. The authors had irradiated a 9:1 mixture of starch and herring oil which they stored in the presence of air for various periods of time before analysis. Under these conditions, which favour oxidation, PUFAs were

unstable even in the non-irradiated controls. However, such a mixture of oil and starch is not representative of any real foodstuff. When herring fillets were irradiated to a dose of 59 kGy, no destruction of PUFAs was observed (115).

When whole grains of rye, wheat and rice were irradiated, no loss of PUFAs was observed in the dose range 0.1–1 kGy, and only small losses occurred at 63 kGy, the highest dose employed (irradiation at ambient temperature in the presence of air) (116). No change was found in the linoleic acid concentration of soya beans with doses up to 100 kGy, whereas 16% of linolenic acid was lost at the highest radiation dose (117).

Peanut kernels irradiated with doses up to 20 kGy and analysed after one year of storage in air at 14 °C showed no significant changes in fatty acid composition. When peanuts were stored at ambient temperature, linoleic acid decreased from 40.2% of total fatty acids in the non-irradiated sample to 39.4% in the 20 kGy-irradiated sample, while linolenic acid decreased from 1.7% to 1.1%; however, it appears unlikely that these small changes were statistically significant (irradiation was apparently undertaken at ambient temperature and in the presence of air) (118). No significant effects on the fatty acid composition of the lipids of chicken meat sterilized by gamma-ray or electron irradiation for the Raltech feeding studies were observed (78).

Taken together, these studies indicate that the irradiation of food in the dose range under consideration has no or only marginal effects on essential fatty acids.

4.5 Minerals or trace elements

Minerals and trace elements are not affected by irradiation, and there is no evidence that the bioavailability of these elements might be adversely affected by irradiation.

4.6 Conclusions

In summary, the macronutrients – proteins, fats and carbohydrates – are not significantly altered in terms of nutrient value and digestibility by irradiation treatment. Among the micronutrients, some of the vitamins are susceptible to irradiation to an extent very much dependent upon the composition of the food and on processing and storage conditions. Retention of the sensory quality of food to be irradiated to doses above 10 kGy will, except in the case of dry products, require irradiation in the absence of oxygen and at cryogenic temperatures, which will also enhance the retention of nutritional quality. From a nutritional viewpoint, irradiated foods are substantially equivalent or superior to thermally sterilized foods.

In assessing which foods are suitable for high-dose irradiation and how much they contribute to the daily supply of vitamins, it appears that thiamine is the only vitamin for which calculation of dietary intakes should be considered, because it is quite sensitive to radiation and because foods that can make an important contribution to the supply of this vitamin, such as pork, are likely candidates for high-dose irradiation processing. It is unlikely, however, that the irradiated foods of this type would constitute a large enough proportion of the diet to compromise the dietary requirement for thiamine.

5. Microbiological considerations

5.1 Introduction

The presence of pathogenic microorganisms represents the most significant hazard in food. A structured risk assessment is widely accepted as a necessary basis for the control of this hazard, and HACCP analyses are widely agreed upon to be the most cost-effective means for control.

The microbiological safety of foods irradiated to doses of less than 10 kGy was reviewed by past expert panels (119, 120), and the conclusions of these panels are in agreement with those of the 1980 Joint Expert Committee (1). Detailed reviews of this subject are also available in the literature (121, 122).

Irradiation of food to doses above 10 kGy may involve: (1) radiation sterilization for safe and shelf-stable high-moisture foods, mainly foods of animal origin, but also complete meals or components of meals (e.g. for immunosuppressed persons or for astronauts) (123); and (2) radiation decontamination of low-moisture products, such as spices, herbs or dried vegetables (124).

Radiation sterilization combines mild heat treatment to inactivate proteolytic enzymes (i.e. heating to an internal temperature of 73–77 °C), vacuum packaging and deep freezing prior to and during radiation processing (123, 125). In the case of low-acid products, this process should deliver a radiation dose sufficient to reduce the population of spores of *Clostridium botulinum* by 10^{12} (the 12D dose) (123, 125) (see section 5.4).

The dose needed for radiation decontamination of dry products lies mainly in the medium-dose range of 3–10 kGy, but in some cases there may be a reason to extend it to 30 kGy. Similarly, a dose lower or higher than 10 kGy may be considered for radiation treatment of some dried vegetable products to improve their rehydration properties or to reduce their cooking times.

The ecological conditions relevant to contaminating microorganisms are different in the high-moisture environment of typical high-dose irradiated foods to those in dried foods and ingredients. Factors relating to microbiological safety should therefore be considered separately for these two applications. Microbiological data on radiation decontamination of animal feed and laboratory animal diets are available and provide information complementary to data on high-dose irradiation of dried foods.

The Study Group reviewed the effects of ionizing radiation on microorganisms and the factors influencing their radiation resistance. It also surveyed the literature on radiation resistance of vegetative bacterial cells, animal parasites, yeasts, mould propagules, bacterial endospores, viruses and preformed microbial toxins. The potential of mathematical modelling of microbial growth and inactivation, with particular reference to modelling inactivation of irradiated bacterial spores, was also considered. The following sections summarize the Group's findings.

5.2 **Effects on microorganisms: factors influencing radiation resistance**

The biological effects of ionizing radiation on cells can be due both to direct interactions with critical cell components and to indirect actions on these targets by molecular entities formed as a result of the radiolysis of other molecules in the cell, particularly by radicals formed from water.

As with other antimicrobial measures, the response of a microbial cell, and hence its resistance to ionizing radiation, depends on:

- the nature and amount of direct damage produced
- the number, nature and lifetime of radiation-generated reactive chemical entities and the inherent ability of the cell either to tolerate radiation damage or to repair it accurately
- the influence of the intracellular and extracellular environments on the above factors.

Therefore, any attempt to categorize or compare the radiation resistance of microorganisms is only meaningful when all related conditions are precisely defined and understood.

Ionizing radiation is capable of causing a variety of chemical changes in microorganisms. It is generally assumed that DNA is the most critical target of ionizing radiation and that the inactivation of microorganisms by ionizing radiation is a result of damage to their DNA.

Ionizing radiation can affect DNA either directly, by energy deposition in this macromolecule, or indirectly, by energy deposition in the surrounding water leading to the formation of diffusive primary

radicals, including hydrogen atoms (H^\bullet), hydroxyl radicals (OH^\bullet) and solvated electrons (e_s^-). The OH^\bullet radical is the most important; OH^\bullet radicals formed in the hydration layer around the DNA molecule are responsible for 90% of the damage. Consequently, in living cells, the indirect effect is especially significant.

The principal effect induced in DNA is chemical alteration to the purine and pyrimidine bases and to the deoxyribose component, resulting in a break in the phosphodiester backbone in one strand of the molecule (single-strand break) and, to a lesser extent (5–10%) to breaks in both strands in close proximity (double-strand break) (126). Both prokaryotes (bacteria) and eukaryotes (moulds and yeasts) are capable of repairing many of the different breaks. It is generally believed that microorganisms that are sensitive to radiation cannot repair double-strand breaks, whereas radiation-resistant species have some capacity to do so. Effects on the plasma membrane appear to play an additional role in radiation-induced damage to cells (127).

The major extracellular environmental factors that influence the survival of irradiated cells (127, 128) are:

- temperature/phase
- the nature of the gaseous environment
- water activity
- pH
- chemical composition of the food.

These extracellular conditions can presumably modify the physical and chemical consequences of intracellular deposition of energy. Bacterial spores appear to be less susceptible to modifying factors than are vegetative cells, because of their specific structure.

It is generally recognized that the radiation survival of microorganisms is not affected appreciably by the rate at which a specific dose is absorbed under practical conditions of food irradiation, except where rate of oxygen replenishment is a factor.

5.2.1 **Temperature/phase**

Elevated temperature treatments, generally in the sublethal range above 45 °C, synergistically enhance the bactericidal effects of ionizing radiation on vegetative cells, particularly when applied simultaneously (129). This is thought to occur because the repair systems that normally operate at or slightly above ambient temperature are damaged at higher temperatures. With respect to bacterial spores, radiation resistance decreases progressively with increasing temperature between 80 °C and 95 °C (130, 131).

Vegetative microorganisms are considerably more resistant to irradiation at subfreezing temperatures than at ambient temperatures (132, 133). This is attributable to a decrease in water activity at subfreezing temperatures (see section 5.2.3). In the frozen state, moreover, the diffusion of radicals is very much restricted. Bacterial spores are less affected by subfreezing temperatures (134, 135); since their core has a low moisture content, no appreciable effect on the already restricted diffusion of radicals would be expected.

5.2.2 **Gaseous environment**

The presence of oxygen increases the lethal effects of ionizing radiation on microbial cells. In anaerobic and wet conditions, the resistance levels of vegetative bacteria may be expected to increase by factors ranging from 2 to about 5 as compared to those in aerated systems (136). Data plotted for cell suspensions irradiated in sealed tubes frequently give a concave survival curve with a resistant “tail”. The latter may represent a shift to anaerobic conditions, because the irradiation of an air-saturated aqueous solution will lead to the consumption of all of the available oxygen in solution after a dose of about 0.5 kGy. If oxygen can be readily resupplied and its uptake into the cell matches or exceeds the rate of depletion, then a resistance corresponding to aerobic conditions should be found.

5.2.3 **Water activity**

Microorganisms are much more sensitive in a high-moisture environment than when the suspending medium is partially or completely dehydrated. In low-moisture conditions, the yield of radicals formed from water molecules by irradiation is much lower and so the level of indirect effects on DNA that they may generate is decreased. The partially dehydrated state of the protoplast of bacterial spores is a major factor in their high radiation resistance. During germination, the water content of the spore protoplast increases, and radiation resistance significantly decreases. Irradiation of food in the frozen state increases the radiation resistance of many vegetative bacteria by a factor of about 2 (133, 137). However, for *Pseudomonas* and *Acinetobacter*, an increase in their radiation resistance by factors of up to 6.7 was reported, while a combination of freezing and anoxia increased resistance by a factor of 8.8. External water activity or freezing has relatively little effect on the radiation resistance of bacterial spores (130), attesting to the impact of the spore coat cortex and substances formed in the forespore stage as protective barriers against the transfer of extracellular components as well as that of the “dry” state of the protoplast.

5.2.4 **pH and chemical composition of the surrounding medium**

Since part of the effect of ionizing radiation on a microorganism is due to indirect action mediated through radicals, the nature of the medium or menstroom in which the microorganisms are suspended obviously plays an important role in determining the dose required for a given microbiocidal effect. The more complex the medium, the greater is the competition by its components for the radicals formed by irradiation within the cell, thus “sparing” or “protecting” the microorganisms.

The radiation resistance of aerobic bacterial spores was practically unaffected in the pH range 5–8, whereas below 5 sensitivity was increased (138).

Some chemical preservatives, such as curing salts, that have an affinity for solvated electrons appear to have a radiation sensitizing effect (139), which is conceivably related to the enhancement of OH[•]-induced changes in DNA.

5.3 **Post-irradiation effects**

It is now a well-established fact that, as in the case of heat-damaged cells, microorganisms that survive irradiation treatment will probably be more sensitive to environmental conditions (temperature, pH, nutrients, inhibitors, etc.) than are untreated cells (140–147). Therefore, it is possible in principle to enhance the microbiological effectiveness of irradiation and reduce the dose required for food preservation, thereby improving product quality, by combining the irradiation treatment with other additives and conditions stressful to microorganisms.

5.4 **Relative radiation resistances**

The cumulative amount of absorbed radiation energy required to inactivate microorganisms in a food depends on their resistance and on the number of them present.

Radiation resistances, even under comparable conditions, vary widely among different microorganisms. There can be differences in inherent resistance from species to species, and even among strains of the same species. Differences in radiation sensitivities within groups of similar organisms are related to differences in their chemical and physical structure as well as in their ability to recover from radiation injury.

It is clear from the survival kinetics of microbial populations subjected to ionizing radiation that the dose required to preserve or decontaminate a food depends on the initial level of the contaminating microorganisms. Early food irradiation research in fact showed that, in the range of populations of practical importance, the rate of radiation inactivation of

microorganisms is not influenced by the initial population, i.e. fractional loss following a particular dose is the same at all population levels (138, 148). Thus, all conditions being equal, it requires a larger dose to inactivate a large number of microorganisms than to inactivate a small number.

Radiation survival is conveniently represented by the logarithm to base 10 of the number of surviving organisms plotted against radiation dose. A linear response on a semilogarithmic plot corresponds to simple exponential kinetics and is quite common for the more radiation-sensitive microorganisms. A survival plot characterized by an initial “shoulder” indicates that equal increments of radiation are more effective at doses above a certain threshold dose level than below that level. The shoulder may be explained by multiple targets and/or certain repair processes being operative at low doses, but then made inoperative at higher doses.

As in the case of response to heat, the response of a microbial population to radiation exposure can be expressed by the dose of irradiation needed to produce a 10-fold reduction in the population of microorganisms (D_{10} -value), associated with the straight line portion of a dose-survival plot, D_{10} being the reciprocal of the slope:

$$D_{10} = \frac{\text{Dose}}{(\log N_0 - \log N)}$$

where N_0 is the initial number of microorganisms and N is the number of microorganisms surviving the radiation dose. For microorganisms with survival plots that include a shoulder, the response can be expressed as the length of the shoulder (L-value) plus the D_{10} -value of the exponential part of the survival curve. Curvilinear survival plots are also often represented by an inactivation dose. It is customary to express inactivation in terms of the reduction in the initial count expressed to the power of 10 (D-value), e.g. a reduction by 10^{12} (12D) for low-acid canned foods.

5.4.1 **Vegetative bacterial cells**

D_{10} -values of vegetative bacterial cells under comparable conditions in non-frozen and frozen foods are listed alphabetically in Tables 6 and 7, respectively (149–166).

It can be seen from Table 6 that *Yersinia*, *Pseudomonas*, *Campylobacter*, *Aeromonas* spp. and the vegetative cells of *Bacillus cereus* are the most radiation-sensitive vegetative bacteria, with D_{10} -values of between 0.04 and 0.20 kGy in non-frozen foods. *Escherichia coli* (including *E. coli* O157:H7) and *Arcobacter butzleri* are also quite radiation-sensitive, with D_{10} -values in the range 0.24–0.40 kGy in non-frozen products.

Table 6
D₁₀ - values of vegetative cells of some foodborne bacteria in non-frozen high-moisture foods

Bacterium	Product	Temp. (°C)	Atmosphere	D ₁₀ (kGy)	Reference
<i>Aeromonas hydrophila</i>	Ground fish	2±1	Air	0.140-0.193	149
	Ground pork	22±1	Air	0.110-0.152	149
<i>Arcobacter butzleri</i>	Roast beef	NS	Vacuum	0.27±0.01	150
	Gravy	NS	Air	0.173±0.157	151
<i>Bacillus cereus</i>	Cauliflower (cooked)	NS	Air	0.181±0.167	151
	Potato (cooked)	NS	Air	0.207±0.099	151
	Ground pork	NS	Air	0.199±0.056	151
	Filet américain	NS	Vacuum	0.19±0.01	150
	Ground beef	18-20	Micro-aerophilic	0.08-0.11	152
<i>Campylobacter jejuni</i>	Ground beef	18-20	Micro-aerophilic	0.14-0.16	152
	Ground beef	0-5	Air	0.161	153
	Ground beef	30±10	Air	0.174	153
	Ground beef (low fat)	4±1	Air	0.175	154
	Ground beef (high fat)	4±1	Air	0.178-0.199	154
	Ground turkey	0-5	Air	0.186	153
	Ground turkey	30±10	Air	0.162	153
	Minced pork	10	Air	0.826	155
	Minced pork	10	CO ₂ :N ₂ (1:3)	0.750	155
	Roast beef	NS	Air	0.586±0.071	151
	Gravy	NS	Air	0.411±0.059	151
	Cauliflower (cooked)	NS	Air	0.528±0.065	151
	Potato (cooked)	NS	Air	0.347±0.054	151
<i>Escherichia coli</i> E.coli O157:H7	Ground beef (low fat)	2	Air	0.36	156
	Mech. deb. chicken	0	Air	0.26±0.01	157
	Mech. deb. chicken	0	Vacuum	0.27±0.01	157
	Ground beef	0	Vacuum	0.27±0.03	157
	Ground beef (low fat)	4±1	Air	0.241	154
	Ground beef (high fat)	4±1	Air	0.251	154

Table 6 (continued)

Bacterium	Product	Temp. (°C)	Atmosphere	D ₁₀ (kGy)	Reference
<i>Listeria monocytogenes</i>	Minced chicken meat	NS	Air	0.417-0.553	158
	Mech. deb. chicken	2-4	Air	0.27-0.77	159
	Minced pork	10	Air	0.573-0.648	151
	Minced pork	10	CO ₂ :N ₂ (1:3)	0.602-0.709	151
	Roast beef	NS	Air	0.644±0.061	155
	Gravy	NS	Air	0.599±0.042	155
	Cauliflower (cooked)	NS	Air	0.564±0.055	155
	Potato (cooked)	NS	Air	0.532±0.047	155
	Ground beef (low fat)	4±1	Air	0.578-0.589	160
	Ground beef (high fat)	4±1	Air	0.507-0.574	160
	Filet américain	18-20	Air	0.45	152
	Ground beef	18-20	Air	0.67	152
	Ground beef (low fat)	2	Air	0.69	152
	Filet américain	18-20	Air	0.49	152
<i>S. enteritidis</i>	Ground beef	18-20	Air	0.66	152
	Filet américain	18-20	Air	0.61	152
	Ground beef	18-20	Air	0.78	152
	Filet américain	18-20	Air	0.37	152
<i>S. stanley</i>	Ground beef	18-20	Air	0.55	152
	Ground beef (low fat)	2	Air	0.59	156
<i>S. typhimurium</i>	Minced pork	10	Air	0.403-0.860	155
	Minced pork	10	CO ₂ :N ₂ (1:3)	0.394-0.921	155
	Roast beef	NS	Air	0.569±0.067	151
	Gravy	NS	Air	0.416±0.058	151
	Cauliflower (cooked)	NS	Air	0.590±0.075	151
	Potato (cooked)	NS	Air	0.464±0.080	151
	Mech. deb. chicken	20	Air	0.52-0.56	161
	Mech. deb. chicken	20	Vacuum	0.52-0.56	161
	Minced chicken	4	Air	0.436-0.502	162
	Minced chicken	4	Air	0.436-0.502	162

Table 6 (continued)

Bacterium	Product	Temp. (°C)	Atmosphere	D ₁₀ (kGy)	Reference
<i>Salmonella</i> spp.	Minced chicken	4	CO ₂	0.436-0.502	162
	Minced chicken	4	N ₂	0.550-0.662	162
	Ground beef (low fat)	4±1	Air	0.621-0.624	154
	Ground beef (high fat)	4±1	Air	0.618-0.661	154
<i>Staphylococcus aureus</i>	Roast beef	NS	Air	0.387±0.056	151
	Gravy	NS	Air	0.360±0.043	151
	Cauliflower (cooked)	NS	Air	0.427±0.055	151
	Potato (cooked)	NS	Air	0.424±0.042	151
	Mech. deb. chicken (in buffered peptone)	0	Vacuum	0.26-0.36	157
	Ground beef (low fat)	4±1	Air	0.437-0.453	160
	Ground beef (high fat)	4±1	Air	0.443-0.448	160
<i>Streptococcus faecalis</i> ^a	Ground beef (low fat)	2	Air	0.57	156
	Minced chicken meat	4	Air	0.651	162
	Minced chicken meat	4	CO ₂	0.702	162
	Minced chicken meat	4	Vacuum	0.697	162
	Minced chicken meat	4	N ₂	0.679	162
	Filet américain	18-20	Air	0.043-0.080	152
	Ground beef	18-20	Air	0.10-0.21	152
<i>Yersinia enterocolitica</i>	Ground beef	25	Air	0.196	163
	Minced pork	10	Air	0.164-0.204	151
	Minced pork	10	CO ₂ :N ₂	0.176-0.187	151

mech. deb. = mechanically deboned; NS = not specified.

^a Now classified as *Enterococcus*.

Staphylococcus aureus, *Salmonella* spp. and *Listeria monocytogenes* are relatively more radiation resistant when compared to other non-sporeforming pathogenic bacteria, with most reported D_{10} -values being in the range 0.4–0.8 kGy in non-frozen food, somewhat similar to the vegetative cells of *Clostridium perfringens*. Relatively radiation-resistant species are *Streptococcus (Enterococcus) faecalis* and *Moraxella phenylpyruvica* with D_{10} -values in the range 0.65–0.86 kGy.

5.4.2 **Radiation-resistant vegetative bacteria**

Vegetative bacteria that are much more radiation-resistant have also been found. Many of them are closely related Gram-negative to Gram-variable, non-sporeforming bacteria designated *Moraxella-Acinetobacter* (M-A), which exhibit a wide range of resistances (167, 168). Some isolates have shown a radiation resistance that appeared to be greater than that of bacterial spores. (Radiation-resistant *Moraxella* isolated by Welch and Maxcy (169) from meat showed a range of D_{10} -values of 2.73–20.4 kGy.) These bacteria appear to be part of the normal flora of meats (170–172) and are not aberrant forms arising from the irradiation process. However, they are not associated with food spoilage except in marine fish and shellfish (173) and are relatively heat sensitive ($D_{70^\circ\text{C}}$ is 5.4 min or less) (169).

For many years it has been known that other non-sporeforming bacteria exist that are more resistant to radiation than are bacterial spores. The first, a red-pigmented coccus, *Micrococcus radiodurans*, was originally isolated by Anderson et al. (170) from ground beef. Its D_{10} -value was found to be 2.5–3.08 kGy when irradiated in raw beef at 5°C (174). In contrast, its heat resistance is low, similar to that of “regular” vegetative bacteria ($D_{60^\circ\text{C}}$ is about 0.75 min) (175). Much of the radiation resistance manifests itself as a shoulder in the survival plot. These highly pigmented radiation-resistant bacteria, however, represent a very low relative proportion of the total number of bacteria (170, 176). Taxonomic studies suggest that the radiation-resistant, red-pigmented, catalase-positive cocci are distinct from conventional *Micrococcus* species and have several characteristics of Gram-negative bacteria (177, 178). Therefore, a new generic name has been proposed, *Deinococcus*, denoting a new family, *Deinococcaceae*.

Deinococcus radiodurans has a highly efficient capacity for repairing damage to DNA induced by both ultraviolet light (179) and ionizing radiation (180). Double-strand breaks in DNA produced by ionizing radiation have been shown specifically to be repairable in this bacterium (181). Redundancy of genetic information in combination with an efficient DNA-repair mechanism could be responsible for the extreme radiation resistance of this organism (182). *Deinococcus radiodurans* has

been subsequently isolated from other sources (183), and other radiation-resistant, morphologically similar species (e.g. *M. radiophilus*) have been reported in studies on irradiated fish products (184, 185).

None of the radiation-resistant micrococci studied is pathogenic (147, 185). The radiation destruction of *Deinococcus radiodurans*, however, can be increased by post-irradiation incubation at 42 °C (186), showing that this organism might not be able to recover from sublethal radiation doses under some environmental conditions.

Strains of a carrot-red, radiation-resistant bacterium called *Pseudomonas radora* have been isolated from rice (187). According to Danish authors (188), these bacteria should perhaps not be classified as *Pseudomonas*. A Gram-negative, red or pink, rod-shaped bacterium has been isolated from animal faeces and freshwater fish (189). The organism, designated as *Deinobacter grandis* gen. nov., sp. nov., has a D₁₀-value of 3.6 kGy when irradiated in phosphate buffer. A similar radiation-resistant, Gram-negative rod has been reported from irradiated pork by Grant and Patterson (190). Its D₁₀-value in pork mince is 5.05 kGy. Numbers observed in pork were quite low (about 100 CFU/g).

In view of their intense carotenoid redness, it has been thought that the pigments concerned, with their high reactivity towards radiation-induced radicals, might contribute, along with an efficient DNA repair capability, to the radiation resistance of these bacteria (191).

5.4.3 **Radiation-resistant vegetative bacteria: relevance to sterilization**

The heat sensitivity of all extremely radiation-resistant, non-sporeforming vegetative bacteria is such that the thermal enzyme inactivation treatment given to food prior to irradiation will destroy or injure most cells (192). Heated cells of *Moraxella-Acinetobacter* and the haemolytic, radiation-resistant *Micrococcus (Deinococcus)* isolated from chicken meat were more sensitive to radiation inactivation and injury than were unheated cells (193, 194). Thus the combined process of heat, irradiation and an unfavourable microenvironment (the procedure involves vacuum packaging and, frequently, addition of sodium chloride and tripolyphosphates as well) would assure that these radiation-resistant cells are unlikely to be a problem in high-dose irradiated goods (176). It is noteworthy that *Moraxella-Acinetobacter*, *Deinococcus radiodurans* and *Pseudomonas radora*, which may be able to survive high irradiation doses, are all markedly sensitive to low solute concentrations (170, 195); consequently, their growth in some foods, particularly in cured meats, could be restricted by lower water activities (145). Actually, no viable radiation-resistant bacteria have been found in properly processed, high-dose irradiated food products, probably owing to a combination of such

Table 7
D₁₀ - values of vegetative cells of foodborne bacteria in frozen foods

Bacterium	Product	Temp. (°C)	Atmosphere	D ₁₀ (kGy)	Reference
<i>Aeromonas</i>	Shrimp paste	-20	Vacuum	0.21	164
<i>hydrophila</i>	Ground fish	-15±2	Air	0.222-0.340	149
<i>Campylobacter</i>	Ground beef	-30	Air	0.315	153
<i>jejuni</i>	Ground beef (low fat)	-16±1	Air	0.235	154
	Ground beef (high fat)	-16±1	Air	0.178-0.199	154
	Ground turkey	-30±10	Air	0.293	153
<i>Escherichia coli</i>	Surface of prawn	-10±2	Air	0.235	165
<i>E. coli</i> O157:H7	Ground beef (low fat)	-16±1	Air	0.39	154
	Ground beef (high fat)	-16±1	Air	0.307	154
<i>Listeria</i>	Shrimp paste	-20	Vacuum	0.70	164
<i>monocytogenes</i>	Ground beef (low fat)	-16±1	Air	0.558-0.610	160
	Ground beef (high fat)	-16±1	Air	0.524-0.575	160
<i>Salmonella enteritidis</i>	Surface of prawn	-10±2	Air	0.49	165
<i>S. typhimurium</i>	Mech. deb. chicken	-20	Air	0.45-0.70	161
	Mech. deb. chicken	-20	Vacuum	0.48-0.79	161
<i>Salmonella</i> spp.	Ground beef (low fat)	-16±1	Air	0.756-0.800	154
	Ground beef (high fat)	-16±1	Air	0.675-0.745	154
<i>Staphylococcus aureus</i>	Surface of prawn	-10±1	Air	0.29	165
	Ground beef (low fat)	-16±1	Air	0.443-0.451	160
	Ground beef (high fat)	-16±1	Air	0.435-0.448	160
<i>Yersinia enterocolitica</i>	Ground beef	-30	Air	0.388	163, 166
<i>Vibrio cholerae</i>	Surface of prawn	-10±2	Air	0.11	165
<i>V. alginolyticus</i>	Shrimp paste	-20	Vacuum	0.19	164
<i>V. fluvialis</i>	Shrimp paste	-20	Vacuum	0.44	164
<i>V. mimicus</i>	Shrimp paste	-20	Vacuum	0.75	164
<i>V. parahaemolyticus</i>	Shrimp paste	-20	Vacuum	0.44	164
<i>V. vulnificus</i>	Shrimp paste	-20	Vacuum	0.30	164

mech. deb. = mechanically deboned.

factors as low initial levels of contamination, heat sensitivity, heat injury and a high dose of irradiation (176).

Table 7 shows that the non-sporeforming pathogens also listed in Table 6 (plus the *Vibrio* species) still remain quite radiation-sensitive in frozen foods. A 10-kGy dose would reduce their populations by at least 12D in the frozen products indicated. Hence, non-sporeforming pathogenic bacteria cannot survive the high-dose irradiation being addressed here.

Comparing Tables 6 and 7, it is worthwhile noting that either freezing of the food or packing the product under vacuum or in an oxygen-free atmosphere generally leads to a smaller increase in the radiation tolerance of bacteria than is found in aqueous model systems.

5.4.4 **Foodborne parasites**

Research into the effects of radiation doses on specific parasites has been reviewed (196–199). The effects of irradiation on fish- and meat-borne parasites are summarized in Table 8 (200).

With respect to fish-borne, snail-borne, or crustacean-borne parasites, liver flukes and *Paragominus* spp. can be controlled by low doses of radiation. In contrast, *Angistrongylus* spp. are relatively radiation resistant. The effectiveness of gamma-ray irradiation in destroying metacercariae of the trematode *Heterophyes* spp. in fish caught in brackish water has been studied in Egypt (201). For its complete destruction at the highest infestation level found in fish, a dose of 7.5 kGy was required. For inactivation of *Anisakis* spp., a dose of 6–10 kGy was required. Irradiation of non-frozen fish as a single treatment with doses higher than 1–2 kGy is not feasible, because of unfavourable quality changes. However, sublethal doses could render the larvae of these parasites non-infectious or non-pathogenic.

Doses below 1 kGy may be effective in controlling the meat-borne parasites listed in Table 8 (198, 202). In the United States, irradiation of pork for trichina control is permitted with a minimum dose of 0.3 kGy and a maximum of 1 kGy (203).

With respect to other parasites that can be transmitted by food, it is worth mentioning the protozoan *Entamoeba histolytica*, which is acquired by humans through consumption of faecally contaminated water or raw fruits and vegetables harbouring the infective cysts, and the dwarf tapeworm *Hymenolepis nana*, which does not need an intermediate host and which is acquired by eating cereals, dried fruits and other foods infested with the larval stage. A dose of 0.25 kGy killed all viable cysts of *Entamoeba histolytica* (204), and 0.37 kGy effectively prevented development of *Hymenolepis nana* to the egg-producing

Table 8
The effect of irradiation on parasites^a

Parasite	Occurrence/mode of infection	Dose (kGy)	Effect of irradiation
Parasites in fish and crustacea:			
<i>Angiostrongylus cantonensis</i>	Parasitic worm found in uncooked molluscs, shellfish	2	Minimum effective dose
<i>Anisakis</i> spp.	Nematode ingested if fish is eaten raw or lightly salted	2-10	Reduces infectivity of larvae
<i>Chlonorchis</i> spp.	Chinese liver fluke, occurs in raw fish	0.15	<i>In vitro</i> minimum effective dose
<i>Gnathostoma spinigerum</i>	Parasitic worm found in raw, undercooked or fermented fish	7	Reduces worm recovery rate in mice
<i>Opisthorchis viverrini</i>	Liver fluke found in contaminated raw, pickled or smoked fish	0.1	<i>In vitro</i> minimum effective dose
<i>Paragonimus</i> spp.	Parasitic worm found in crabs and crayfish in Asia	0.1	<i>In vitro</i> minimum effective dose
Parasites in meat:			
<i>Cysticercus bovis</i> (<i>Taenia saginata</i> , in meat)	Tapeworm found in uncooked or undercooked beef, causes taeniasis	0.3	Preliminary minimum effective dose
<i>Cysticercus cellulosae</i>	Tapeworm found in pork	0.3	Preliminary minimum effective dose
<i>Toxoplasma gondii</i>	Consumption of undercooked meat or poultry; or cooked with infected animals	0.7	Minimum effective dose for fresh pork
<i>Trichinella spiralis</i>	Nematode occurs in raw or inadequately cooked pork	0.3 0.3-1	Minimum effective dose FDA permitted dose to control trichina in pork

^a Adapted from Wilkinson and Gould (200) with the permission of the publisher.

stage (205). The eggs of *Ascaris lumbricoides* worms enter the human body with contaminated raw vegetables. A dose of approximately 1–1.5 kGy applied to infective (i.e. embryonated) *Ascaris* eggs was effective in preventing the development of viable larvae in the lungs of guinea pigs (206).

With respect to radiation sterilization/stabilization of foods, these data show that the combination of sequential heating and freezing plus the high radiation doses required inactivates even the most resistant parasites.

5.4.5 Yeasts

The radiation resistance of some yeasts in phosphate buffer is given in Table 9 (207). Since many yeasts have relatively low resistance to ionizing radiation, with D_{10} -values within the range 0.1–0.5 kGy, a dose of 5 kGy would be expected to reduce their numbers by at least 10D (200). However, some yeasts are much more tolerant. A radiation-resistant strain of *Saccharomyces cerevisiae* var. *ellipsoideus* studied by Stehlik and Kaindl (208) had a D_{10} -value as high as 3 kGy when irradiated at about 20 °C. The inactivation rate increased greatly as the temperature was raised, so that at 45 °C the D_{10} -value fell to about 0.5 kGy.

Japanese authors isolated other radiation-resistant yeasts: *Pullularia (Aureobasidium) pullulans* (209) and *Trichosporon oryzae* nov. sp. (207). The dormant blastospores of the latter were more sensitive to gamma-ray irradiation than vegetative cells. In these studies, some other *Trichosporon* species such as *T. capitatum* and *T. pullulans* were also relatively radiation resistant.

The survival plots of yeasts vary in shape from sigmoidal or biphasic to simple linear, and are dependent upon the irradiation menstroom. As a result of the extensive shoulder, doses as high as about 5 kGy may be required to achieve a ten-fold reduction of the initial count (e.g. in the case of *Trichosporon cutaneum* in sausage meat) (210).

Table 10 indicates the dose required for preventing the growth of some yeasts within a specified post-irradiation incubation time (211–213).

Some such yeasts, owing to their radiation tolerance and if present in high enough numbers initially, may survive in some medium-dose irradiated foods (1–10 kGy) (1) and could become the major – though harmless – flora. This situation has been observed, for example, in certain chill-stored crabmeat irradiated to about 4 kGy (214). If air is

Table 9
Radiation resistance of some yeasts in phosphate buffer (0.067 mol/l)^a

Yeast	Condition	Induction dose (shoulder) (kGy)	D_{10} -value (kGy)
<i>Candida</i> sp. V3-1	Air-bubbling	0	0.32
<i>Saccharomyces cerevisiae</i> 52A	Air-bubbling	0.32	0.36
<i>Pullularia pullulans</i>	Air-equilibrium	0.2	1.6
<i>Trichosporon</i>	Air-bubbling	2.5–3.0	1.2
<i>oryzae</i> nov. op. R1	Air-equilibrium	3.0–3.5	1.6

^a Adapted from Ito et al. (207) with the permission of the publisher.

Table 10
Gamma-radiation resistance of some species of yeast irradiated at ambient temperature

Yeast	Initial cell number per ml	Irradiation medium	Irradiation dose required to prevent growth (kGy)	Post-irradiation medium and other conditions	Reference
<i>Candida crusei</i>	10^7	Phosphate buffer	5.5	Malt extract agar, 27 °C for up to 15 days	211
<i>C. tropicalis</i>	10^7	Phosphate buffer	10	Malt extract agar, 27 °C for up to 15 days	211
<i>Cryptococcus albidus</i>	$0.6-2.5 \times 10^6$	Grape juice	10	Grape juice, 17 °C, for 21 days	212
<i>Debaryomyces klöckeri</i>	$0.6-2.5 \times 10^6$	Grape juice	7.5	Grape juice, 17 °C for 21 days	212
<i>Pullularia pullulans</i>	10^7	Phosphate buffer	20	Malt extract agar, 27 °C for up to 15 days	211
<i>Rhodotorula glutinis</i>	$0.6-2.5 \times 10^6$	Grape juice	10	Malt extract agar, 27 °C for up to 15 days	212
<i>Saccharomyces carlsbergiensis</i>	$1.5-3.0 \times 10^6$	Grape juice	15	Grape juice, 25 °C for 19 days	213
<i>S. cerevisiae</i>	$1.5-3.0 \times 10^6$	Grape juice	18	Grape juice, 25 °C for 19 days	213
<i>S. rosei</i>	$1.5-3.0 \times 10^6$	Grape juice	15	Grape juice, 25 °C for 19 days	213
<i>Sporobolomyces pararoseus</i>	$0.6-2.5 \times 10^6$	Nutrient broth	5.0	Nutrient broth, 25 °C for 21 days	212
<i>Torulopsis stellata</i>	$1.5-3.0 \times 10^6$	Grape juice	10	Grape juice, 25 °C for 19 days	213

excluded, e.g. by vacuum or modified atmosphere packaging, the lactic acid bacteria tend to outgrow any surviving yeasts and constitute the eventual spoilage flora (215).

Since yeasts are heat sensitive and non-poisonous, even the most radiation-tolerant yeast is of no significance to high-dose irradiated foods.

5.4.6 **Mould propagules**

Since it is difficult to determine cell numbers from the mass of hyphae-producing moulds, their radiation sensitivity is usually not expressed in the form of a D_{10} -value, except for conidia spores whose numbers can be determined.

The age of mould cultures can have a considerable influence on their radiation sensitivity (216). As in the case of bacteria, results from radiation resistance studies of fungi may also be influenced by the post-irradiation environmental conditions, e.g. the growth medium (217).

Table 11 indicates the dose required for preventing the growth of mould spores within a specified post-irradiation incubation time (212, 217, 218).

Table 12 compares estimated D_{10} -values for some commonly occurring moulds (219). While the radiation resistances of conidiospores of *Aspergillus* spp. and *Penicillium* spp. are similar to those of the less radiation-tolerant vegetative bacteria, the D_{10} -values for both *Curvularia geniculata* and *Alternaria alternata* were at least three times greater. Nine of the 14 species listed in Table 12 apparently exhibited higher sensitivities to electron-beam irradiation than to gamma-ray irradiation. Chelack et al. (220) working with *Aspergillus alutaceus* var. *alutaceus* (formerly *A. ochraceus*) also reported higher D_{10} -values when gamma-ray irradiation was used.

A D_{10} -value of 0.4 kGy was reported for gamma-ray irradiated aqueous suspensions of *Aspergillus parasiticus* (NRRL 3145) (221). Ascospores of the heat-resistant mould, *Byssoschlamys fulva*, had a D_{10} -value of 1.2 kGy in apple juice (222).

D_{10} -values of mould conidia are higher when they are irradiated in the dry state (223). When barley was inoculated with conidia of the toxigenic fungus, *Aspergillus alutaceus* var. *alutaceus* (10^6 conidia per gram), ochratoxin production was not detected after 3.0 kGy of electron and 4.0 kGy of gamma-ray radiation (221, 224).

Table 11
Radiation resistance of some species of moulds irradiated at ambient temperature

Mould	Initial number of conidia per ml	Irradiation medium	Ionizing radiation	Irradiation dose required to prevent growth (kGy)	Post-irradiation medium and other conditions	Reference
<i>Apergillus flavus</i>	4 x 10 ⁶	0.1% peptone + 0.1% Tween 80	Electrons	1.6	Czapek agar, 25°C for 5 days	217
<i>A. niger</i>	Not given	Malt extract agar	Gamma-rays	2.5	Malt extract agar, 27°C for 10 days	212
<i>A. parasiticus</i>	1 x 10 ⁶	Water	Gamma-rays	1.6	Autoclaved rice, 26–28°C for 10 days	218
<i>Alternaria</i> spp.	Not given	Malt extract agar	Gamma-rays	6.0	Malt extract agar, 27°C for 10 days	212
<i>Botrytis cinerea</i>	Not given	Malt extract agar	Gamma-rays	5.0	Malt extract agar, 27°C for 10 days	212
<i>Cladosporium</i> spp.	Not given	Malt extract agar	Gamma-rays	6.0	Malt extract agar, 27°C for 10 days	212
<i>Penicillium viridicatum</i>	4 x 10 ⁶	0.1% peptone + 0.1% Tween 80	Electrons	1.4	Czapek agar, 25°C for 5 days	217

Webb et al. (225) reported the destruction of *Aspergillus flavus* strains with a dose of 3.5 kGy in ground corn samples containing 12.5–23% moisture. Some species of *Hormodendrum* and *Verticillium* as well as *Rhizopus nigricans* in corn containing 12.5% moisture survived a dose of 10 kGy; however, in corn containing 23% moisture, *R. nigricans* was inactivated by only 2.5 kGy. Ito et al. (226) concluded from studies on *Aspergillus* spoilage of rice, maize, milo and wheat that the “sterilization doses” for spoilage moulds of cereal grains should be 5–6 kGy. No growth of *Aspergillus ochraceus* (NRRL 3174) occurred from 0.3-cm mycelial discs on a synthetic medium when they were exposed to a 3 kGy dose of gamma-rays (227).

In a gamma-ray irradiation study with wheat, 3 kGy was required to completely inactivate *Aspergillus*, *Rhizopus* and *Absidia*, whereas a dose of 10 kGy was required for complete inactivation of *Alternaria* and *Fusarium* (228).

With respect to high-dose food irradiation, the survival of fungal contamination cannot be expected in high-moisture foods. Tables 11 and 12 show that the mould genera *Aspergillus* and *Penicillium*, including the toxigenic species, are among the more radiation-sensitive moulds. If a high burden of some fungi such as *Alternaria alternata*, *Cladosporium cladosporioides* or *Culvularia* spp. was present in dry foods or dry ingredients, small numbers of them might survive irradiation to dose levels above 10 kGy (229). However, proper primary processing and pre-irradiation storage of dry commodities should prevent the develop-

Table 12
Comparison of D₁₀-values of mould spores in aqueous suspensions, irradiated at ambient temperature^a

Mould	Gamma-irradiated (kGy)	Electron-irradiated (kGy)	Values not significantly different (P<0.005, Student <i>t</i> -test)
<i>Aspergillus echinulatus</i>	0.319	0.241	
<i>A. fumigatus</i>	0.276	0.198	
<i>A. glaucus</i>	0.250	0.243	x
<i>A. niger</i>	0.245	0.199	
<i>A. ochraceus</i>	0.209	0.198	x
<i>A. versicolor</i>	0.282	0.234	x
<i>Penicillium aurantiogriseum</i>	0.236	0.194	x
<i>P. cyclopium</i>	0.397	0.290	
<i>P. granulatum</i>	0.239	0.201	
<i>P. roqueforti</i>	0.416	0.341	
<i>P. verrucosum</i>	0.266	0.208	
<i>P. viridicatum</i>	0.333	0.265	x
<i>Curvularia geniculata</i>	1.798	1.193	
<i>Alternaria alternata</i>	2.409	1.099	

Reproduced from Blank and Corrigan (219) with the permission of the publisher.

^a Survivors were estimated by plating in potato dextrose agar with incubation at 25 °C for 5 days.

ment of such high levels of contamination and should exclude an increase in moisture content to levels that would allow any fungal growth.

5.4.7 **Bacterial spores**

Bacterial spores belonging to the genera *Clostridium* and *Bacillus* are of major concern in the microbiology of high-dose irradiated, high-moisture, low-acid foods because several sporeforming species pose serious health hazards, while many others are associated with food spoilage. In general, spores are highly resistant to radiation, heat and chemicals.

Radiation resistance values of aqueous unbuffered (“water”) or buffered suspensions of spores important in food preservation are listed in Table 13, and indicate that radiation resistance differs among strains to an extent not evident at the species level (128, 136, 142, 230–237). Whenever a large number of strains was tested (e.g. *C. botulinum* type A), the variations from strain to strain with the same serotype were large. The variability within an individual strain of *C. botulinum* was investigated in detail by Grecz et al. (238). It is important in this connection to remember that D_{10} -values are influenced by various irradiation conditions (e.g. temperature, oxygen level, suspending medium) and by the composition of the recovery medium (239). The correlation of DNA repair mechanisms in spores with the shoulder portion of their survival plots has been studied by Grecz et al. (240).

The radiation resistance values of bacterial spores in deep-frozen foods are given in Table 14 (130, 242–245).

Clostridium botulinum type A and B spores are apparently the most resistant and thus of greatest concern in the radiation sterilization of food, whereas the less radiation-resistant type E spores are important in low-dose irradiation of foods, particularly fishery products. While types A and B will not grow below 10 °C, type E strains may grow and produce toxin at refrigeration temperatures (3–4 °C) (127). The proteolytic strains of types A, B and F produce a conspicuous off-odour, whereas the nonproteolytic strains of types B, E and F produce none, so spoilage by the latter may easily be missed by the consumer.

The spores of the other food poisoning *Clostridium* species, *C. perfringens*, are less radiation resistant than those of *C. botulinum*, and the food poisoning is less severe than botulism.

The lack of correspondence between heat and radiation resistance is illustrated by the fact that the highly heat-resistant spores of *B. stearothermophilus* and of thermophilic anaerobic spores are relatively radiation sensitive, their D_{10} -values being well below those of the highly radiation-resistant spores of *C. botulinum* type A (237, 245).

Table 13

Radiation resistance of aqueous or buffered suspensions of spores important in food preservation^a

Organism	D ₁₀ (kGy)	“Shoulder” (kGy)	Irradiation medium	Reference
Anaerobes:				
<i>Clostridium botulinum</i> type A				
Resistant strains				
33	3.4	3.5	Buffer	230
62	2.7	3.5	Buffer	142
Medium-resistant strains				
37	2.0	0.7	Buffer	142
36	1.9	0.7	Buffer	231
Sensitive strains				
1192y	1.4	0–1.0	Water	232
NCTC 7272	1.2		Water	233
<i>C. botulinum</i> type B				
Resistant strains				
53	3.3	4.0	Buffer	231
41	2.1	1.6	Buffer	142
Medium-resistant strain				
9	1.6	1.8	Buffer	142
Sensitive strains				
213	1.1	0.9–1.0	Water	232
51	1.2	0.0	Buffer	142
<i>C. botulinum</i> type D				
	2.2	2.5–3.5	Buffer	232
<i>C. botulinum</i> type E				
Medium-resistant strains				
Beluga	1.9	1.5	Buffer	142
Alaska	1.7	2.1	Buffer	142
16/63	1.6	2.5–3.5	Water	232
1304	1.7	0.0	Water	234
Sensitive strains				
Beluga	0.8	0.7	Water	232
8	0.8	0.7	Buffer	136
V.H.	1.3	1.4	Buffer	142
Minneapolis	0.8	2.0	Buffer	136
<i>C. botulinum</i> type F				
	2.5	2.5–3.5	Water	232
<i>C. perfringens</i>				
type A	1.2	2.5–3.5	Water	232
type B	1.7	2.5–3.5	Water	232
type C	1.8	2.5–3.5	Water	232
type E	1.2	2.5–3.5	Water	232
<i>C. sporogenes</i>				
PA 3679/S ₂	2.2	2.5–3.5	Water	232
NCTC 532	1.6	2.5–3.5	Water	232
Aerobes:				
<i>Bacillus cereus</i>	1.6	2.0	Water	235
<i>B. subtilis</i>	0.6		Saline (with 5% gelatin)	234
	2.4	0.0	Buffer	236
<i>B. stearothermophilus</i>	1.0	0.0	Buffer	237

^a Adapted from Grecz et al. (127) and from Goldblith (275) with the permission of the publishers.

Table 14

Radiation resistance of bacterial spores in deep-frozen foods

Organism	Food	Irradiation temperature (°C)	D ₁₀ -value (kGy)	Reference	
<i>Bacillus cereus</i>	Mozzarella cheese	-78	3.6	241	
	Ice cream	-78	4.1	241	
	Yoghurt	-78	4.0	241	
<i>Clostridium botulinum</i>	type A	Beef	-196	5.9-7.1	130
	type A + B cocktail	Corned beef	-30±10	1.0-2.6	242
		Pork sausage	-30±10	0.7-1.8	242
		Codfish cake	-30±10	0.7-3.3	242
		Beef	-30±10	2.5-3.6	243
		Roast products	-29	4.0-6.8	244

Early studies suggested that certain combination treatments have advantages for inactivation of bacterial spores. The most promising are the combinations of radiation with heat and/or food additives (e.g. sodium chloride). The order in which irradiation and heating followed each other may have played an important role in the inactivation. When spores were heated first and then irradiated, there seemed to be little or no difference in their total inactivation. However, when spores were irradiated first, their subsequent heat resistance was very remarkably decreased (246, 247). This sensitization to heat inactivation by irradiation depends on the initial heat resistance (248) as well as on the suspending substrate (246, 248)

Indirect effects of irradiation seem to sensitize spores to heat inactivation more effectively than direct effects. This follows from observations that prior irradiation of *C. botulinum* 33A spores under conditions extremely conducive to indirect effects (unfrozen in buffer at 0–25 °C) led to 2.5 times greater sensitivity to heat than prior irradiation under conditions of primarily direct action (frozen, –25 to –196 °C) (249). Sensitization to heat inactivation increases as the irradiation dose is increased (250).

Foods irradiated to high doses are of acceptable sensory quality only when irradiated at subfreezing temperatures (–30 ± 10 °C) (251). Therefore, the effect of irradiation temperature on spore survival in the range +20 °C down to the temperature of the liquid nitrogen (–196 °C) is of great importance.

Ingram and Thornley (252) studied the effect of temperature on the inactivation of *C. botulinum* spores in minced pork meat irradiated with either electrons (2 MeV) or gamma-rays *in vacuo* and concluded that there were no significant differences between the computed

sterilizing doses at 0 and -75°C . El-Bisi et al. (253) using gamma-rays found that irradiation temperature had little effect on the rate of inactivation of *C. botulinum* spores in cooked, vacuum-canned, cubed beef below -80°C . In contrast, Grecz et al. (254) noted progressively decreasing radiation resistance of *C. botulinum* spores in vacuum-canned, ground beef with increasing irradiation temperature between -196°C and $+95^{\circ}\text{C}$.

The process of radiation sterilization is based on the 12D destruction of the most radiation-resistant spores of *C. botulinum* (255). The 12D concept is based on studies by Esty and Meyer on the heat resistance of *C. botulinum* spores (256) and is used in determining the time and temperature needed to establish the safety and efficacy of thermal canning. To ensure that irradiation would provide the same margin of safety as that of thermal canning, the 12D concept proposal by Schmidt (257) was adopted by the 1964 Joint FAO/IAEA/WHO Expert Committee (7) and has received acceptance worldwide as the minimum required dose (MRD) for radiation sterilization. Because D_{10} -values may vary with the food product formulation, it may be necessary to determine the MRD experimentally for each food as it would be processed commercially.

The MRD is usually determined by inoculated pack studies. Since the radiation resistance of a microorganism can vary with the food substrate, the typical procedure involves using prototype foods, vacuum-packed in cans, that have been inoculated with a “cocktail” of 10 different strains of *C. botulinum*, approximately 10^6 spores per strain, to give a total of 10^7 spores per can. The cocktail inoculum represents the most resistant strains as well as strains of intermediate resistance. The procedure also entails irradiating 100–1000 cans per dose in the dose range 5–50 kGy in 4–5 kGy increments (more cans per dose being used at the higher doses where fewer survivors are expected) at $-30 \pm 10^{\circ}\text{C}$, incubating the cans at $+30^{\circ}\text{C}$ for 6 months, and analysing for swelling, botulin toxin and recoverable *C. botulinum*. The 12D dose can be estimated using the minimum experimental dose required for sterilization based on non-swollen and non-toxic samples, e.g. by the binomial confidence limits method and extreme-value statistics (258–260).

Table 15 shows the 12D doses for typical radiation sterilized foods as determined in various inoculated pack studies by the United States Army Natick Research and Development Laboratories, Natick, Massachusetts (242, 243, 260–263). It is clear from these data that appropriate 12D doses for *C. botulinum* in enzyme-inactivated cured meats are lower than those for enzyme-inactivated non-cured meats (264).

Table 15

12D irradiation doses for *Clostridium botulinum* in various foods

Food ^a	12D value (kGy)	Reference
Bacon	26.5–28.7	243
Beef	41.2	260
Chicken	42.7	261
Corned beef	25.7	242
Ham	31.4	262
Pork	43.7	261
Pork sausage	23.9	242
Codfish cake	31.7	263

^a Irradiated at $-30 \pm 10^\circ\text{C}$, except bacon which was irradiated at $5\text{--}25^\circ\text{C}$.

5.4.8 Irradiation above 10 kGy in combination with other processes

As with shelf-stable, cooked, cured meat products, which do not require 12D heat inactivation of *C. botulinum*, there is interest in certain non-sterile products made shelf-stable by a combination of treatments, including irradiating to less than a 12D dose, where irradiation in combination with growth-inhibiting factors (e.g. sporestatic additives, reduction of pH and/or water activity) ensures microbiological safety and shelf-stability (265). For such products, the dose equivalency level for total protection (i.e. spore destruction plus inhibition of the survivors) needs to be determined on the basis of the probability of growth and toxigenesis of *C. botulinum*; similarly, the inhibiting factors needed to prevent the outgrowth of surviving spores must be quantified (266). For this concept published by Ingram and Roberts (267), an experimental method has been developed by Hauschild (268). This method has been used for assessing the safety of shelf-stable canned cured meats (269) and extensively discussed by Lund (270). The application of this method for comparing the efficiency of combined processes inclusive of irradiation has been reported (271, 272).

5.4.9 Viruses

Viruses are more radiation resistant than bacteria; however, their resistance may vary by as much as ten-fold depending on a number of factors, particularly the concentration of organic materials in the suspending medium, the temperature during irradiation and the degree of dehydration (127).

Table 16 illustrates this resistance in the case of coxsackievirus B2 (273). In contrast to suspensions in water, no trend in D_{10} -values with temperature was seen when the virus was suspended in raw and in cooked ground beef. Apparently, there was efficient radical scavenging by

Table 16

Gamma-radiation resistance of coxsackievirus B2 in water and cooked ground beef

Suspending medium	Irradiation temperature (°C)	D ₁₀ -value (kGy)	99% Confidence limits
Water	0.5	1.4	1.0-2.1
	-90	5.3	4.7-6.2
Ground beef	16	7.0	6.6-7.4
	0.5	7.6	7.4-7.9
	-30	6.8	6.3-7.2
	-60	7.8	7.2-8.4
	-90	8.1	7.7-8.5

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proteins or other substances in the ground beef to eliminate or reduce the indirect effect of irradiation.

Massa (274) found D₁₀-values for foot-and-mouth disease virus of 4.8 kGy and 6.26 kGy when irradiated in liquid medium and in the dry state, respectively. A later report (275) cites a personal communication from British who determined a D₃₇-value (dose to reduce initial population by 63%) for this virus of about 5 kGy when irradiated frozen at -60 °C. It has been estimated that carcasses of animals infected with foot-and-mouth virus can be rid of infective viruses with a dose of 20 kGy (276).

Small human viruses (e.g. hepatitis A) are probably about as radiation-resistant as the foot-and-mouth disease virus (277).

The D₁₀-value of poliovirus type 1 was found to be approximately 4 kGy in experimentally contaminated oysters (278). A 99% reduction of poliovirus in fish fillets was observed after an irradiation dose of 6 kGy (279).

Simultaneous application of heat (47 °C) and radiation (i.e. thermoradiation) more readily destroyed the poliovirus in wastewater sludge than irradiation at 20 °C (280). This thermoradiation treatment became even more effective as the concentration of suspended solids was lowered. Other experiments with T1 bacteriophage (281) and Newcastle disease virus (282) showed a marked increase of radiation sensitivity at temperatures higher than 50 °C.

Cliver (283) reported on studies intended to determine whether or not viruses contaminating foods would be likely to mutate as a consequence of irradiating the food. Four enteroviruses were selected as models: poliovirus 1 (strain CHAT), coxsackievirus A-9 (strain Borek), coxsackievirus B-2 (undesignated strain), and virus 6 (strain D'Amori). These were treated with gamma-ray doses of 2-5 kGy. On the basis of

these experiments, the author concluded that the weight of evidence indicated that any significant virus mutation is unlikely.

A number of criteria would need to be met before the mutagenic changes taking place in radiation-damaged viruses could prove harmful (127): (1) the virus must retain its ability to penetrate into a suitable host cell; (2) the host cell nucleases must not degrade the damaged virus; (3) the host-cell replicative and repair mechanism must be subverted into the production of a mutant; (4) mutation must be towards increased pathogenicity rather than loss of pathogenicity; and (5) mutants must be produced in sufficiently large numbers to present a public health danger.

Available data on thermal inactivation in solid foods show that a 3D reduction in viruses would be achieved in 1 min at 71 °C or in 6 sec at 75 °C (260, 284).

Considering the high dose irradiation of high-moisture foods, the associated heat pretreatment to inactivate proteolytic enzymes along with the sterilization dose used would inactivate foodborne viruses (260, 276). Radiation-decontaminated dry commodities might contain some viable viruses, but high-dose irradiated products would in any case have fewer viruses than either non-irradiated or low/medium-dose irradiated products.

5.4.10 **Preformed microbial toxins**

Bacterial toxins appear to be rather radiation resistant in complex suspending media or in food. The toxin of *C. botulinum* type E was found by Skulberg (285) to have a D_{10} -value, when irradiated in a rich bacteriological medium, of about 21 kGy; the D_{10} -value for type A toxin was nearer to 40 kGy. Depending on the suspending medium and the assay used, D_{10} -values of <0.4–36 kGy have been estimated for *C. botulinum* type A neurotoxin and 2–200 kGy for staphylococcal enterotoxin A (286–289).

Mycotoxins already formed are also resistant to irradiation (290–292). Van Dyck et al. (293) reported an almost complete destruction of aflatoxin B1 by irradiation to 20 kGy, but they studied aqueous solutions of a commercial aflatoxin preparation. Aflatoxins in food are much more resistant. Temcharoen and Thilly (294) reported that irradiation of aflatoxin-contaminated peanut meal required a dose of 50 or 100 kGy to eliminate the effect of aflatoxin on a bacterial test system. Irradiation to 1–10 kGy eliminated 75–100% of the effect expressed as toxicity but not as mutagenicity. Doses as high as 180 kGy have been reported to degrade only 10% of aflatoxin in a dry environment (295). Simultaneous treatment with hydrogen peroxide and gamma-ray irradiation resulted

in a synergistic inactivation of aflatoxin B1 in contaminated groundnuts (296).

Pure ochratoxin dissolved in methyl alcohol was found to be stable even to 75 kGy (227).

These results are consistent with the inability of the toxin molecules to compete against the high concentration of other constituents for the primary radicals formed in a medium. It can therefore be concluded that, owing to the high radiation resistance of preformed microbial toxins, irradiation should only be used in conjunction with good manufacturing and storage practices to prevent the proliferation of toxigenic microorganisms and the associated production of toxin prior to irradiation. The same requirement already exists for other food preservation processes owing to the heat stability of mycotoxins and many bacterial toxins.

5.5 Modelling the inactivation of irradiated spores

Predictive modelling is potentially of great value to the food industry; validated predictive models would allow confident development of safe new foods, processes and distribution systems with less need for time-consuming and costly inoculated pack studies or challenge tests (265). Over the last ten years, developments in the modelling of microbial growth have resulted in models with sound mathematical and biological bases (297–301). The introduction and integration of irradiation as a factor into these new predictive microbiological models and their associated databases, which are becoming available for worldwide food industry use, would be of great importance.

Thayer and co-workers have recently published empirical regression equations for the survival of various vegetative pathogenic bacteria following irradiation in the low-dose range as a function of dose and irradiation temperature (149, 161, 302).

As far as high-dose irradiation of food is concerned, further systematic studies would be required, in which environmental factors important in food are systematically varied across the range of interest, in order to develop models for radiation inactivation of bacterial spores, particularly those of *C. botulinum*. In principle, such studies could determine the correlation, if any, between the D_{10} -value for *C. botulinum* and such parameters as irradiation temperature, pH, sodium chloride content, water activity and content of preservatives, e.g. nitrite. The existing extensive literature, although invaluable for demonstrating the microbiological safety of high-dose irradiated food in general, is not sufficiently detailed to provide all the information needed for modelling.

A mathematical model for microbial destruction by radiation has been suggested by Brynjolfsson (303). It takes into account radiation effects on genetic constituents and the ability of the microorganism to repair damage to its DNA. The model agrees well with experimental data, including those that show survivor–dose curves with a shoulder or sigmoidal shape.

5.6 Conclusions

The Study Group concluded that high-dose irradiation presents no special microbiological problems. Issues such as selective destruction of microorganisms and potential mutations, which were scrutinized carefully in connection with low- and medium-dose irradiation, are of less concern or irrelevant at radiation doses higher than 10 kGy.

The main potential application of high-dose food irradiation, namely radiation processing of precooked and prepackaged high-moisture food, renders the food shelf-stable and microbiologically safe.

In the case of high-dose irradiation to decontaminate dry commodities (with doses up to 30 kGy), low numbers of radiation-resistant microbial cells may survive. However, these survivors cannot grow in the low-water environment of dry spices or dried vegetables. These cells are radiation-damaged and have an increased sensitivity to heat, salt and changes in pH.

For high-dose food irradiation, as with other methods of food manufacturing, it is important to use raw materials of good microbiological quality, provide adequate packaging, follow proper processing procedures, maintain adequate record-keeping, follow good personal hygiene and sanitation practices, and handle the processed foods appropriately during distribution.

It is important to note that, in establishing food irradiation technologies, the steps of risk assessment for hazardous microbiological agents had already been followed well before the modern concept and terminologies of risk assessment were developed. The four stages of risk assessment have therefore been fully addressed:

- (1) hazard identification (i.e. the most radiation-tolerant pathogenic microorganisms);
- (2) hazard characterization (i.e. toxin formation by *C. botulinum*, the most critical biological agent);
- (3) exposure assessment (i.e. the efficacy of processing for inactivation of spores through the application of the 12D concept, whose very high safety margin takes all reasonable uncertainties into consideration, and the D-equivalency concept in the case of combined antimicrobial agents and/or treatments); and

- (4) risk characterization (i.e. the severity and likelihood of intoxication, which is extremely low for sterilization processes).

Accordingly, the irradiation of foods to doses above 10 kGy to achieve shelf-stability of high-moisture foods, such as meals and meal components, and to decontaminate low-moisture products, such as spices, herbs and dried vegetables is deemed safe:

- The radiation-sensitive vegetative forms of all pathogenic bacteria will be inactivated by more than 10^{12} -fold.
- The doses necessary to achieve a 10^{12} -fold reduction in numbers of the most radiation resistant of the pathogenic sporeformers, *C. botulinum*, are well established for different strains of the microorganism and in different types of foods (about 45 kGy for uncured meats; about 30 kGy for cured meats). These data clearly define the minimum doses required for the irradiation of high-moisture foods and take into account the small uncertainties due to strain-to-strain variation.
- The radiation tolerance and heat sensitivity of viruses are such that high-dose irradiation will result in high levels of inactivation because of the combined effects of irradiation and the associated heat treatment that is employed to inactivate enzymes.

From the point of view of risk assessment, high-dose irradiation is no different from thermal processing in producing shelf-stable, microbiologically safe foods; both processes have outstanding records of safety.

6. Toxicological considerations

6.1 Introduction

The safety of high-dose irradiated foods has been evaluated in many feeding studies conducted over the past four decades that have involved a variety of laboratory diets and food components given to humans and a broad cross-section of animal species, including rats, mice, dogs, quails, hamsters, chickens, pigs and monkeys. These investigations, which have included subacute, chronic, reproductive, multigeneration and carcinogenicity studies, have been conducted under a variety of experimental protocols and have covered a range of doses. In addition, a large number of evaluations for mutagenicity have been conducted in *in vitro* and *in vivo* systems. In terms of extrapolation to humans, the data derived from animal studies are especially relevant because of the composite nature of the food materials used and the manner in which the diets were administered.

The database assembled over decades by a large number of divergent research organizations provides important cumulative information. Thus, available data now appear to be adequate for evaluating the safety of high-dose irradiated foods for human consumption.

6.2 **Relevant factors**

Irradiation conditions are important factors in determining the quality of all irradiated foods and in evaluating the potential toxicological effect of irradiating food and feed. For example, the presence of oxygen during irradiation results in the production of peroxides and other potentially toxic oxidative agents that may affect the nutritional quality and palatability of the diet. Removal of oxygen before irradiation, especially from lipid components in food, limits the production of these compounds. Foods can be canned or packaged in a vacuum or under nitrogen to limit the oxygen content in the food. Enzyme inactivation by heating is also important, because irradiation is not an effective enzyme inactivator. However, dry or dried foods with a low water content, such as spices, can be irradiated to high doses in the presence of oxygen with minimal degradation. Ideal or proper food irradiation conditions limit the oxygen content and require that the foods (with the exception of dry products) be irradiated at freezing temperatures, to minimize unwanted chemical reactions that affect odour and taste. Dose is especially relevant to both quality and safety. The dose used should exceed the minimum needed to achieve commercial sterilization.

The method of preparation of the food is important in establishing that irradiated foods are wholesome and palatable. As in any food processing procedure, the use of high-quality raw materials is a precondition for high-quality processed products. Criteria for determining irradiation conditions depend on the amount of water, protein, lipid and carbohydrate in the food, and on the temperature and the atmospheric conditions during irradiation. The United States Army Natick Laboratories minimized unwanted side effects by heat-inactivating the proteolytic enzymes, vacuum-packing the food in a can or flexible pouch, and irradiating these foods at freezing temperatures (304, 305).

6.2.1 **Radiation sources and irradiation dose**

Researchers have used a variety of radiation sources to irradiate food for animal testing, with gamma-ray and machine sources (electron beam, X-ray) being the primary choices.

Spent fuel rods have also been used. In the 1950s and 1960s, as part of its review of radiations sources, the United States Army determined that the neutrons present in spent nuclear fuel rods were insufficient to produce

measurable amounts of radioactivity above background (306). Food was usually canned and frozen prior to irradiation, then stored at room temperature for a minimum of three months before use in animal studies. However, these foods were not enzyme-inactivated prior to irradiation and so foods with high water activity would break down and oxidize upon storage at room temperature for extended periods (307–310; A. Brynjolfsson, personal communication). The control samples were usually frozen until used in the feeding studies.

The dose rate, which determines the duration a product must be exposed to accumulate the target dose, varied depending on source and source strength. Irradiation conditions become increasingly important the longer the process takes. The doses reported later in this section in Tables 17–26 are average doses, although in some early studies, the reported dose may have been the minimum dose, the average dose, or the actual absorbed dose range.

6.3 Toxicity studies in animals

The safety of irradiated foods has been evaluated using animal feeding studies with a wide variety of species and protocols over the last four decades. These studies have focused primarily on teratogenic, mutagenic and carcinogenic end-points. It is difficult to identify any other food processing technology the safety of which has been supported by so many animal toxicity studies.

The studies discussed in this section include all types of food. Researchers and regulatory scientists decided that worst-case scenarios should include a high percentage of irradiated food in the test diets (311). These diverse studies have been grouped in three ways in the series of tables that follows (312–422). The first group indicates the source of radiation (spent fuel rods, gamma-ray, machine) and processing conditions used in preparing the test foods for the animal feeding studies (Tables 17–20) and mutagenicity studies (Tables 21 and 22). The second group of tables lists the animal studies according to study type (Tables 23–26). The last group summarizes the studies by food type and test species (Tables 27–32); the summary includes information on the food used in the diet, the percentage of irradiated food in the diet, the dose, the process conditions, and the number of animals. It also provides some comments about the study. If the author did not specify radiation conditions or other specific information, the notation NS (not specified) is used. In addition, the studies are coded as NHDIR (negative for high-dose irradiation effect), PEND (possible effect of nutrition or diet) or PEHDIR (possible effect of high-dose irradiation).

(Text continues on page 119)

Table 17
Sources of radiation – rat studies

Source	Packaging	Irradiation	Atm., temp.	Shipping	Storage	Author/reference
Spent fuel rods:						
A, D, S	Canned			Frozen	8 °C	Mead & Griffith (312)
A, D, S	Canned			Frozen	8 °C	Teply & Kline (313)
A, D, S	Canned			Frozen	8 °C	Read et al. (314)
A, D, S	Canned			Frozen	8 °C	Malhotra & Reber (315)
A, D, S	Canned			Frozen	8 °C	Malhotra & Reber (316)
A, D, S	Canned			Frozen	8 °C	Malholtra, Reber & Norton (317)
A, D, S	Canned			Frozen	8 °C	Blood et al. (318)
A, D, S	Canned			Frozen	rt	Read et al. (319)
A, D, S	Canned			Frozen	8 °C	Read, Kraybill & Witt (320)
A, D, S	Dry packed in cans			Frozen	rt	Tinsley, Bone & Bubl (307); Bone (321)
A, D, S	Canned chicken and green beans			Frozen	rt	Richardson (322)
A, D, S	Canned			Frozen	rt	Richardson, Ritchey & Rigdon (323); Rigdon (324)
A, D, S	Canned chicken stew, raw shredded cabbage, plastic bags in fibre drum			Frozen	rt/refrig.	Phillips, Newcomb & Shanklin (325)
D	Canned			Frozen	rt	Radomski et al. (326)
D	Peaches canned in syrup			Frozen	rt	Tinsley, Bone & Bubl (327)
A, D, S	Ground pork canned under vacuum			Frozen	rt 3–8 months	Bubl & Butts (328); Bubl (329)

Table 17 (continued)

Source	Packaging	Irradiation	Atm., temp.	Shipping	Storage	Author/reference
A, D, S	Canned			Frozen	rt 3–8 months	Brin, Ostashever & Kalinsky (330)
A, D, S	Canned cooked shrimp Oranges	Surface irradiated with electrons at Michigan State University		NS	rt 3–6 months	Phillips, Newcomb & Shanklin (331) Phillips, Newcomb & Shanklin (331)
A, D, S	Canned			Frozen	rt	Paynter (332)
Gamma-rays:						
Cobalt-60	NS	0.69 kGy/h	NS		NS	Becker et al. (333)
	Vacuum packed in cans, frozen	NS	Frozen		rt	McGown, Lewis & Waring (334)
	PE bags	50 kCi,	NS		10 °C for up to 2 months	Barna (335)
	Plastic bags, paper cartons	50 kCi,	NS			Saint-Lébe (336)
	Plastic bags, paper cartons	0.5 kGy/h	NS			IFIP (337)
	NS	50 kCi,	NS			
	PE bags	0.5 kGy/h	Air, ambient		25–29 °C	Aravindakshan et al. (338)
	NS	NS	NS		NS	Metwalli (339)
	Canned, stored -40 °C	NS	-30 °C		rt	van Logten, Berkvens & Krocs (340)
						van Logten et al. (341)
Caesium-137	NS	1.9 kGy/h	NS		NS	Rojo & Fernandez (342)

Table 17 (continued)

Source	Packaging	Irradiation	Atm., temp.	Shipping	Storage	Author/reference
Machine:	Electron	Vacuum-packed	Frozen		rt	McGown, Lewis & Waring (334)
		in pouches, frozen				
	1/4-inch slabs, frozen	10 MeV, 5 μ s pulse, 180 pulse/s	Frozen		8 °C	Teply & Kline (313)
		NS				
	Oil in bottle PE bags	NS	refrig.		rt	Teply & Kline (313)
		10 MeV, 10 min	NS			
	NS	1 MeV	25 °C		NS	Lang (344)
		(van der Graaf)				
	Fresh in PE bags	3 MeV	NS		Frozen	Verschuuren, van Esch & van Kooy (345)

A = Idaho Falls, ID, United States of America, pool storage; Atm. = atmosphere; D = Dugway, UT, United States of America, hot cell; NS = not specified; PE = polyethylene; refrig. = refrigerated; rt = room temperature; S = Aiken, SC, United States of America; temp. = temperature.

Table 18
Sources of radiation – mouse studies

Source	Packaging	Irradiation	Atm., temp.	Shipping	Storage	Author/reference
Spent fuel rods:	Canned			Frozen	5 °C first	McKee et al. (346);
					6 months, rt to end	Dixon et al. (347)
	Canned Canned		NS	Frozen	rt 3–9 months	Deichmann (348);
					rt 3–9 months	Radomski et al. (349)
A, D, S	Canned Canned Canned Canned			Frozen	rt	Calandra & Kay (350)
					8 °C	Teply & Kline (313)
					rt	Monsen (351–353)
					rt	Thompson et al. (354, 355)
Gamma-rays:						
Cobalt-60	Heated, canned	NS	Frozen		rt	Raltech Scientific Services (356, 357)
					NS	Maffei, Mazzali & DeSantis (358)
	NS	6 kGy/h 50 kCi, 0.5 kGy/h	NS	NS	NS	Biagini et al. (359)
					NS	Saint-Lébe (336)
	NS	1.8 kGy/h, ambient	NS	Ambient	NS	Porter & Festing (360)
					NS	Bugyaki et al. (361)
Caesium-137	Plastic bags, paper cartons	NS	NS		NS	Saint-Lébe (336)

Table 18 (continued)

Source	Packaging	Irradiation	Atm., temp.	Shipping	Storage	Author/reference
Machine: Electron	Heated, vacuum-packed in pouches	10 MeV	Frozen		rt	Raltech Scientific Services (356, 357)
	1/4-inch slabs, frozen	NS	Frozen		8 °C	Teply & Kline (313)
	Oil in bottle	NS	refrig.		rt	Teply & Kline (313)

A = Idaho Falls, ID, United States of America, pool storage; Atm. = atmosphere; D = Dugway, UT, United States of America, hot cell;
NS = not specified; refrig. = refrigerated; rt = room temperature; S = Aiken, SC, United States of America; temp. = temperature.

Table 19
Sources of radiation – dog studies

Source	Packaging	Irradiation	Atm., temp.	Shipping	Storage	Author/reference
Spent fuel rods:						
A, D, S	Bacon, canned/ cabbage, raw shredded, in plastic bags in fibre drum			Frozen/ refrig.	rt/refrig.	Hale, Schroeder & Sikes (362)
A, D, S	Canned			Frozen	rt	Reber et al. (363, 364)
A, D, S	Canned			Frozen	rt	Clarkson & Pick (365)
D	Canned			Frozen	rt	Deichmann (366); Radomski et al. (326, 367)
A, D, S	Canned			Frozen	rt	Blood et al. (367)
A, D, S	Canned			Frozen	rt	Larson et al. (368)
A, D, S	Canned			Frozen	rt	McCay & Rumsey (369–371)
Gamma-rays:						
Cobalt-60	Heated, canned	NS	Frozen		rt	Raltech Scientific Services (372)
	Fresh pork packed in plastic pouches	NS	NS		NS	Cheng & Zhang (373)
	Plastic bags	NS	NS		NS	Smid, Dvorak & Hrusovsky (374)
Machine:						
Electron	Canned	12 MeV	Frozen		rt	Loosli et al. (376)
	Heated, vacuum-packed in pouches	10 MeV	Frozen		rt	Raltech Scientific Services (373)

A = Idaho Falls, ID, United States of America, pool storage; Atm. = atmosphere; D = Dugway, UT, United States of America, hot cell;
 NS = not specified; refrig. = refrigerated; rt = room temperature; S = Aiken, SC, United States of America; temp. = temperature.

Table 20
Sources of radiation – miscellaneous animal feeding studies

Source	Packaging	Irradiation	Atm., temp.	Shipping	Storage	Author/reference
Spent fuel rods:						
A, D, S	Peaches canned in syrup			Frozen	rt	Blood et al. (376)
	Oranges	Surface irradiated with electrons				
Gamma-rays:						
Cobalt-60	Heated, canned	NS	Frozen		rt	Raltech Scientific Services (379)
	NS	NS	NS		NS	Strik (380)
	Glass bottles, cardboard boxes	300 kCi bottle, 100 kCi feed	Ambient		NS	Takigawa, Danbara & Ohyama (381)
Machine:						
Electron	NS	10 MeV, 250 mA, 0.9 cm/s	40-50°C		NS	Koch et al. (377); Döllstädt et al. (378)
	Heated, vacuum-packed in pouches	10 MeV	Frozen		rt	Raltech Scientific Services (379)

A = Idaho Falls, ID, United States of America, pool storage; Atm. = atmosphere; D = Dugway, UT, United States of America, hot cell;
 NS = not specified; rt = room temperature; S = Aiken, SC, United States of America; temp. = temperature.

Table 21
Sources of radiation – mutagenicity studies *in vitro*

Source	Packaging	Irradiation	Atm., temp.	Storage	Author/reference
Gamma-rays:					
Cobalt-60	Plastic film bags	17 kGy/h 6.5 kCi	4 °C, ambient	1, 2, 7, 14, 21 days	Joner, Underdal & Lunde (382)
	Sealed ampoules		O ₂ enriched, 25 °C	NS	Neimand et al. (383)
	NS		NS	NS	Bugyaki, Lafontaine & Moutschen-Dahmen (384)
	Powder form in individual containers, packed in plastic 13-cm diam. containers	4.5 kGy/h	Ambient		Vijayalaxmi (385)
	Plastic film	NS	4 °C, ambient	1, 7, 14 days	Joner & Underdal (386)
	Unsealed plastic pouches in metal cans	NS	Ambient	NS	Münzer & Renner (387)
	NS	NS	Aerobic, ambient	Ambient	Central Food Research Institute (388)
	NS	NS	Aerobic, ambient	NS	Farkas, Andrassy & Incze (389)
	400-ml beaker	3.6 kGy/h	25 °C	NS	Schubert et al. (390)
	NS	NS	NS	rt or 5 °C several months ^a	Shaw & Hayes (391)
Solution	3.3 kGy/h	NS	NS	Bradley, Hall & Trebikock (392)	
N ₂ flush, frozen, seal; dry ice	4.5 kGy/h, 10 min	Air	6-8 °C up to 25 weeks	Aiyar & Rao (393)	
Machine:					
Electron	NS	10 MeV	NS	NS	Münzer (394)

Atm. = atmosphere; NS = not specified; rt = room temperature; temp. = temperature.

^a 2% solution concentrated to 20% for shipment, diluted with saline before use.

Table 22
Sources of radiation – mutagenicity studies *in vivo*

Source	Packaging	Irradiation	Atm., temp.	Storage	Author/reference
Gamma-rays:					
Cobalt-60	Canned, frozen	3 MCi	Frozen	rt	Mittler (395)
	Canned	NS	Frozen	NS	Luskin (396)
	Canned	NS	Frozen	rt	Raltech Scientific Services (397)
	PE bags	57 Gy/min	Air, ambient	25 °C	Varma et al. (398–401)
	NS	NS	NS	NS	Moutschen-Dahmen, Moutschen & Ehrenberg (402)
	NS	8.5 kGy/h	NS	NS	Johnston-Arthur et al. (403)
	NS	2.4 kGy/h	NS	4–6 °C	Chauhan et al. (404)
	NS	2.4 kGy/h	NS	3–4 weeks	Chauhan et al. (405)
	NS	11–11.5h	Ambient	37–38 °C	Leonard, Wilcox & Schietcatte (406)
	250 g pellets in PE sacks	NS	Ambient	22–24 °C	Johnson-Arthur et al. (407)
	NS	NS	NS	NS	Anderson et al. (408)
	NS	14 kCi, 70 min	NS	NS	Chopra (409)
	NS	NS	Aerobic, ambient	NS	Central Food Research Institute (388)
	NS	3.1 kGy/h	NS	Fed 8–18 days after irradiation	Chaubey et al. (410)
NS	NS	Aerobic, ambient	NS	Farkas & Andrassy (411)	
NS	NS	Aerobic, ambient	NS	Farkas, Andrassy & Incze (389)	
NS	0.5 kGy/h	NS	Fed 5–9 days after irradiation	Barna (412)	
NS	3.6 kGy/h	Ambient	25 °C	Schubert et al. (390)	

Table 22 (continued)

Source	Packaging	Irradiation	Atm., temp.	Storage	Author/reference
	NS	4.5 kGy/h	N ₂ , 10 min, seal	6-8°C for up to 25 weeks	Aiyar & Rao (393)
	Plastic bags	180 krad/h	N ₂ flush	NS	Bugyaki et al. (361)
	NS	NS	Ambient, N ₂ flush	NS	Tanaka et al. (413)
Caesium-137	NS	4 kGy/h	rt	rt	Bernades et al. (414)
Machine:					
Electron	Vacuum-packed in pouches	10 MeV	Frozen	rt	Mittler (395)
	Vacuum-packed in pouches	NS	Frozen	rt	Lusskin (396)
	Vacuum-packed in pouches	NS	Frozen	rt	Raltech Scientific Services (397)
	NS	10 MeV	NS	NS	Eriksen & Emborg (415)
	NS	10 MeV, 10 min	NS	NS	Münzer & Renner (416)
	Open aluminium trays	10 MeV	NS	NS	Renner (417)
	NS	10 MeV	NS	NS	Münzer & Renner (418)

Table 22 (continued)

Source	Packaging	Irradiation	Atm., temp.	Storage	Author/reference
	NS	10 MeV, 10 min	NS	NS	Renner et al. (421)
	Unsealed plastic pouches in metal cans	10 MeV, 10 min	Ambient	NS	Münzer & Renner (387)
X-ray	Medium in lucite vials	25 MeV electrons (600 mA) on tungsten converter, 10 kGy/min	NS	Used immediately or 3 weeks	Rinehart & Ratty (419)
	Medium in lucite vials	250 kV _p , 15 mA, 50 Gy/min	NS	Used immediately or 3 weeks	Rinehart & Ratty (419)
	Sucrose solution in lucite vials	25 MeV electrons (600 mA) on tungsten converter, 10 kGy/min	NS	Used immediately or 3 weeks, diluted to 10% with medium	Rinehart & Ratty (420)
	Herring sperm DNA in lucite vials	250 kV _p , 15 mA, 50 Gy/min	NS	Used immediately or 3 weeks, diluted to 10% with medium	Rinehart & Ratty (420)

Atm. = atmosphere; NS = not specified; PE = polyethylene; rt = room temperature; temp. = temperature.

Table 23
High-dose irradiation study types – rat studies

Study type	Duration	Author/reference
Subchronic	90 days	Malhotra & Reber (315)
	8 and 9 weeks	Malhotra & Reber (316)
	8 and 14 weeks	Malhotra, Reber & Norton (317)
	8–12 weeks	Read et al. (319)
	8–12 weeks	Read, Kraybill & Witt (320)
	54 days	McGown, Lewis & Waring (334)
	200 days	Rojo & Fernandez (342)
	84 days	Brin, Ostashever & Kalinsky (330)
	280 days	Lang (344)
	84 days	Verschuuren, van Esch & van Kaay (345)
	90 days	van Logten et al. (340)
	120 days	Metwalli (339)
Reproduction	Teratology 15 days	IFIP (338)
Carcinogenesis	2 years	Teply & Kline (313)
	2 years	Bone (321)
	2 years	Teply & Kline (313)
	2.5 years	van Logten et al. (341)
Combined carcinogenesis and reproduction	2 years	Mead & Griffith (312)
	2 years	Teply & Kline (313)
	2 years	Read et al. (314)
	2 years	Blood et al. (318)
	2 years	Becker et al. (333)
	2 years	Richardson (323)
	2 years	Richardson, Ritchey & Rigdon (323); Rigdon (324)
	2 years	Phillips, Newcomb & Shanklin (325)
	2 years	Tinsley, Bone & Bubl (307); Bone (321)
	2 years	Barna (335)
	Lifetime,	Saint-Lèbe (336)
	3 generations	
	2 years	Radomski et al. (326)
	3 years	Renner & Reichelt (343)
	2 years	Tinsley, Bone & Bubl (327)
	2 years	Bubl & Butts (328); Bubl (329)
	2 years	Phillips, Newcomb & Shanklin (331)
2 years	Aravindakshan et al. (338)	
2 years	Paynter (332)	

Table 24
High-dose irradiation study types – mouse studies

Study type	Duration	Author/reference
Subchronic	60 days	Maffei, Mazzali & DeSantis (358)
Chronic	14 months 19 months	Teply & Kline (313) Monsen (351–353)
Reproduction	Repro. and teratology 20 days Lifetime, 3 generations Repro. and teratology 200 days	Raltech Scientific Services (356) Saint-Lèbe (336) Porter & Festing (360)
Carcinogenesis	750 days 12–28 months 2 years 730 days	McKee et al. (346); Dixon et al. (347) Deichmann (348); Radomski et al. (349) Calandra & Kay (350) Raltech Scientific Services (357)
Combined carcinogenesis and reproduction	730 days 300 and 600 days, lifetime Lifetime, 3 generations	Biagini et al. (359) Thompson et al. (354, 355) Bugyaki et al. (361)

Table 25
High-dose irradiation study types – dog studies

Study type	Duration	Author/reference
Subchronic	25 weeks	Reber et al. (363)
	90 days	Smid, Dvorak & Hrusovsky (374)
Chronic	2 years	Hale, Schroeder & Sikes (362)
	2 years	Deichmann (366); Radomski et al. (326)
	2 years	Blood et al. (367)
	104 weeks	Larson et al. (368)
	90 weeks and 2–3 years	McCay & Rumsey (369–371)
	4 years	Cheng & Zhang (373)
Reproduction	104 weeks	Reber et al. (364)
	Repro. and teratology, 3 years	Loosli et al. (375)
	104 weeks	Clarkson & Pick (365)
	36 and 40 months	Raltech Scientific Services (372)

Table 26
High-dose irradiation study types – miscellaneous animal feeding studies

Study type	Test species	Duration	Author/reference
Subchronic	Japanese quail	26 days	Koch et al. (377); Döllstädt et al. (378)
	Pigs	16 weeks, 90 days	Strik (380)
	Chicks	5 weeks	Takigawa, Danbara & Ohyama (381)
Chronic	Rhesus monkeys	24 months	Blood et al. (376)
Reproduction	Hamsters, reproduction and teratology	5 days	Raltech Scientific Services (379)
Combined carcinogenesis and reproduction	Pigs	3 generation reproduction	Strik (380)

Table 27
Food type – rat studies

Food type (% in diet) ^a	Study type/ duration	Irradiation dose (kGy) ^b	Process conditions	Group	Animals per group	Comments	Author/ reference
Bacon (b) (35%)	2 years P, F ₁ , F ₂ generations	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; foods canned, shipped frozen; irradiated at 60 °F; stored at rt	9 groups in 3 x 3 matrix with (b) and fruit compote (f)	15M, 15F for C, 5M, 5F for all other groups; Repro: 9M, 9F for C; 3M, 3F for other groups	NHDIR/PEND. Sprague-Dawley. Study done in replicate. Longevity decreased in 4th generation. No carcinogenicity. (See also under fruit compote.)	Mead & Griffith (312)
Beef (35%)	90 days	C = 0 VH = 55.8	Spent fuel rods; foods canned, shipped frozen; irradiated at 60 °F; stored at rt	C and VH dose groups with Met and Tes in diet	10M	NHDIR/PEND. Sprague-Dawley, castrated males. Investigated effects of various methionine (Met) and testosterone (Tes) concentrations in irradiated diet on mortality due to internal haemorrhaging; effect not related to irradiation of diet.	Malhotra & Reber (315)
Beef (35%)	9 weeks (3 exp), 8 weeks (1 exp)	C = 0 VH = 55.8	Spent fuel rods; foods canned, shipped frozen; irradiated at 60 °F; stored at rt	C and VH dose groups with Met added to diet	Approx. 10M	NHDIR/PEND. Sprague-Dawley. Mortality in VH groups compared to controls. Methionine supplementation decreased prothrombin time and mortality of irradiated diet. Adults more susceptible to haemorrhagic diathesis than weanlings.	Malhotra & Reber (316)
Beef (35%)	14 weeks (exp I), 8 weeks (exp II)	C = 0 VH = 55.8	Spent fuel rods; foods canned, shipped frozen; irradiated at 60 °F; stored at rt	C, VH, exp I, 9 groups; exp II, 5 groups	7M	NHDIR/PEND. Sprague-Dawley. Mortality reduced by vitamin K and DL-methionine.	Malhotra, Reber & Norton (317)

Table 27 (continued)

Food type (% in diet) ^a	Study type/ duration	Irradiation dose (kGy) ^b	Process conditions	Group	Animals per group	Comments	Author/ reference
Beef (35%)	2 years P, F ₁ -F ₃ generations	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; foods canned, shipped frozen; irradiated at 60 °F; stored at rt	C, H, VH	27M, 27F	NHDIR. Sprague-Dawley. No treatment- related effects in growth, haematological change, food efficiency, reproduction, mortality, gross pathology, or histopathology.	Blood et al. (318)
Beef (35%); raw frozen (rf); raw stored (rs) at 70 or 100 °F; cooked stored (cs) at 70 °F.	8-12 weeks	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; canned meats, shipped frozen; irradiated at 60 °F; stored at rt	cs, C, H, VH; rf, C, H, VH; rs-70, C, H, VH; rs-100, C, H, VH	10M	NHDIR/PEND. Sprague-Dawley, males only. Increase in liver cytochrome oxidase in animals fed rt stored beef or pork, whether raw or cooked. (See also under pork.)	Read et al. (319)
Beef stew (35%)	2 years P, F ₁ -F ₃ generations	C = 0 H = 27.9 VH = 55.8	Spent fuel rods (Dugway)	C, H, VH	25M, 25F	NHDIR. Osborn-Mendel (beef stew); Sprague-Dawley (milk). No significant difference between groups. (See also under evaporated milk.)	Radomski et al. (326)
Beef, fresh ham, haddock, turkey, bacon, corn, spinach, beets, snap beans, peaches, strawberry, bread, cereal bar and powdered milk (14 food items, 35% each)	8-12 weeks	C = 0 H = 30 VH = 60	Spent fuel rods; foods canned, shipped frozen; irradiated at 60 °F; stored at rt	C, H, VH	10M	NHDIR. Holtzmann or Sprague-Dawley, males. Decrease in growth in high- dose peaches group attributed to high sucrose level (commercially prepared sucrose syrup).	Read, Kraybill & Witt (320)
Butter fat (4%); butter fat (53 g), skimmed milk powder (147 g), ground whole wheat (1000 g), salt (20 g), and vitamins A and D	2 years P, F ₁ , F ₂ generations	C = 0 H = 16.8	⁶⁰ Co 0.69 kGy/h; storage NS	C, H	6M, 6F for P; 16M, 16F for F ₁ and F ₂	NHDIR/PEND. Sherman. No significant effect in reproductive performance. Slightly decreased growth rates in F ₁ and F ₂ generations attributed to peroxidative loss of nutrients.	Becker et al. (333)

Table 27 (continued)

Food type (% in diet) ^a	Study type/ duration	Irradiation dose (kGy) ^b	Process conditions	Group	Animals per group	Comments	Author/ reference
Cabbage (ca) (35%) stored in ice-packed fibre drums, at 34 °F, 70% RH	2 years P, F ₁ -F ₃ generations	C = 0 H = 27.9 VH = 55.8 (ca.2.8 and 5.6)	Spent fuel rods; raw, shredded (ca), plastic bags in fibre drums (Dugway)	Repro: chicken stew (cs) and (ca) groups in 3 x 3 matrix	9M, 9F for C; 3M, 3F for all other groups	NHDIR. Charles-River, Sprague-Dawley derived albino rats. Elevated levels of sucrose in duodenal tissue of young rats not attributed to irradiation of diet. (See also under chicken stew.)	Phillips, Newcomb & Shanklin (325)
Carrot (35%): control stored at 0 °F; irradiated turned dark, sometimes surrounded by jelly-like substance, smelled acid	2 years P, F ₁ , F ₂ generations	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; carrots sliced, dry- packed in cans; stored at rt	C, H, VH	25M, 25F	NHDIR. Wistar. No consistent effect associated with diet. Caloric density noted to be very low.	Tinsley, Bone & Bubl (307); Bone (321)
Chicken (35%): Frozen (f), Thermal (t), Gamma (g), Electron (e)	54 days	C = 0 VH = 59	⁶⁰ Co: vacuum- packed in cans, frozen; 10 MeV electrons: 5-µs pulse, 180 pulse/s; vacuum-packed in pouches, frozen	C and VH	12M, 12F	NHDIR/PEND. Charles-River rats. 14-16 days thiamine depletion followed by 27 days repletion. Rats repleted with control or irradiated chicken diets (f, t, g, e). No difference among groups in growth or erythrocyte transketolase response (sensitive to dietary thiamine levels).	McGown, Lewis & Waring (334)
Chicken (ch) (35%), synthetic diet	2 years P, F ₁ -F ₃ generations	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; canned and shipped frozen; irradiated at 60 °F; stored at rt	C, H, VH	10M, 10F for P; 20F for F ₁ -F ₃	NHDIR. 30-year colony at Texas Station ^c . Synthetic diet 4 generations, no significant difference; chicken diet 4 generations, no significant difference; congenital blindness not related to diet. Pathology changes not associated with diet.	Richardson, Ritchey & Rigdon (323); Rigdon (324)

Table 27 (continued)

Food type (% in diet) ^a	Study type/duration	Irradiation dose (kGy) ^b	Process conditions	Group	Animals per group	Comments	Author/reference
Chicken (ch) (35%) and green beans (gb) (35%); synthetic diet	2 years P, F ₁ -F ₃ generations	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; canned (ch) and (gb) shipped frozen; irradiated at 60 °F; stored at rt	Repro: (gb) and (ch) groups in 3 x 3 matrix	9M, 9F for C in P, F ₁ and F ₂ ; 3M, 3F for all other groups	NHDIR. 30-year colony at Texas Station ^c . F ₁ rats in VH gb and C ch groups had the poorest fertility; all VH gb and H ch groups of 3 generations fertile, therefore not related to diet.	Richardson (322)
Chicken stew (cs) (35%); control cs received frozen, stored frozen	2 years P, F ₁ -F ₃ generations	C = 0 H = 27.9 VH = 55.8 cabbage (ca) 2.8 and 5.6)	Spent fuel rods; canned (Dugway)	Repro: (cs) and cabbage groups in 3 x 3 matrix	9M, 9F for C; 3M, 3F for all other groups	NHDIR. Charles-River, Sprague-Dawley derived albino rats. Elevated levels of sucrose in duodenal tissue of young rats not attributed to irradiation of diet. (See also under cabbage.)	Phillips, Newcomb & Shanklin (325)
Corn (co) (35%); control co received frozen, stored frozen	2 years P, F ₁ , F ₂ generations	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; canned, irradiated at 60 °F; stored at rt	3 control groups, 3 x 3 matrix	10M, 10F in 11 groups for 2-year study; 6M, 6F for repro study in P	NHDIR. Charles-River Wistar. No significant differences in 2-year or reproduction groups.	Paynter (332)
Evaporated milk (35%)	2 years P, F ₁ -F ₃ generations	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; (Dugway)	C, H, VH	25M, 25F	NHDIR. Osborn-Mendel (beef stew); Sprague-Dawley (evaporated milk). No significant difference between groups. (See also under beef stew.)	Radomski et al. (326)
Fruit compote (f) (35%)	2 years P, F ₁ , F ₂ generations	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; foods canned, shipped frozen; irradiated at 60 °F; stored at rt	9 groups in 3 x 3 matrix with (b) and (f)	15M, 15F for C; 5M, 5F for all other groups for 2-year study; 9M, 9F in C; 3M, 3F in others for repro study	NHDIR/PEND. Sprague-Dawley. Study done in replicate. Longevity decreased in 4th generation. No carcinogenicity.	Mead & Griffith (312)

Table 27 (continued)

Food type (% in diet) ^a	Study type/ duration	Irradiation dose (kGy) ^b	Process conditions	Group	Animals per group	Comments	Author/ reference
Green beans (gb) (35%) synthetic diet	2 years P, F ₁ -F ₃ generations	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; canned gb shipped frozen; irradiated at 60 °F; stored at rt	C, H, VH	10M, 10F for P; 20F for F ₁ -F ₃	NHDIR. 30-year colony at Texas Station. Synthetic diet 4 generations, no significant difference; chicken/ (323); green bean diet 4 generations, no significant difference; congenital blindness not related to diet. Pathology changes not associated with diet. (See also under chicken.)	Richardson, Ritchey & Rigdon (323); Rigdon (324)
Laboratory diet (100%); autoclaved (auto) or irradiated (irrad)	Lifetime; 3 genera- tions	C = 0 Auto = 0 VH = 44	⁶⁰ Co: paper cartons, plastic bags Auto: 120 °C, 20 min	C, auto, irrad	140M, 140F	NHDIR. Summary of experience with autoclaved or radiation sterilized diets.	Saint-Lébe (336)
Laboratory diet (100%)	200 days	VH = 50	¹³⁷ Cs 1.9 kGy/h; storage NS	0, 20, 30, 90, 200 days	10	NHDIR/PEND. Reversible chromosomal changes.	Rojo & Fernandez (342)
Laboratory diet (100%); wheat (53.5%), gram (16%), skimmed milk powder (10%), shrimp (4%), vegetables (2.5%), sesame oil (6%), sucrose (6%), salt (2%); fed after 3-4 weeks storage at 4-6 °C	2 years P, F ₁ -F ₄ generations	C = 0 L = 2 H = 25	⁶⁰ Co 2.4 kGy/h in air at ambient temperature, 25-29 °C	Chow, C, L, H	12M, 24F for repro study	NHDIR. Wistar. No significant differences between controls (stock diet and non- irradiated) and irradiated groups.	Aravindak- shan et al. (338)
Laboratory diet (100%); autoclaved at 110 °C, autoclaved at 120 °C, for 15 min (auto)	90 days	C = 0 VH = 50	⁶⁰ Co NS	C, auto, VH	15M, 15F	NHDIR. Wistar. Repro: no effect on reproduction parameters. 90 days with F ₁ . No treatment-related histopathological effects.	van Logten et al. (340)

Table 27 (continued)

Food type (% in diet) ^a	Study type/ duration	Irradiation dose (kGy) ^b	Process conditions	Group	Animals per group	Comments	Author/ reference
Laboratory diet (100%): casein (8.5%), skimmed milk (9.4%), potato starch (50%), wheat flour (16.5%), sucrose (5%), sunflower oil (6%), choline chloride (0.1%), salt mixture (3.5%) and vitamin mixture (1%)	120 days	C = 0 H = 25 VH = 45	⁶⁰ Co NS stored in PE bags	C, H, VH	50M, 50F	NHDIR. Sprague-Dawley. Investigated effects of diet on liver function.	Metwalli (339)
Laboratory diet (100%): wheat (18%), barley (17.5%), rye (14%), oat (14%), maize (4%), sunflower seed (8%), alfalfa meal (2%), fishmeal (5%), casein (5%), skimmed milk powder (4%), yeast (4%), salt (0.5%) and vitamin/ mineral premix (4%)	2 years multigenera- tion P 24 months F _{1b} 13 months F _{2b} 9 months F _{1-F3} 1 month	C = 0 H = 25 VH = 45	50 kCi ⁶⁰ Co 0.5 kGy/h; 7-8 kg PE bags; stored at <10 °C for up to 2 months	C, H, VH	50M, 50F in P; 30M, 30F in F _{1-F3} (300 in P, 5014 in F _{1-F3})	NHDIR. Wistar. No adverse or pathological effects in parameters studied: feeding, body weight, reproduction, haematology, blood chemistry, vitamin metabolism, ophthalmology, parasitology, intestinal flora, health condition and behaviour, findings in mortality, gross pathology, histopathology, oncogenesis, and longevity.	Barna (335)
Milk powder (35%) (positive and negative controls for radicals)	3 years P, F _{1-F5} generations	C- = 0 C+ = 25 VH = 45	10 MeV electrons; NS; stored for 10 min	C-, C+, VH	P 120 F ₁ 72 F ₂ 54 F ₃ 72 F ₄ 60 F ₅ 78	NHDIR. Sprague-Dawley. High level of radicals present when fed. No indication of toxic or carcinogenic effects.	Renner & Reichelt (343)
Mixed: bacon (8.75%), beef (8.43%), haddock (19.15%), ham (4.82%), powdered milk (5.56%), beets (15.45%), green beans (8.39%), cereal (3%) and peaches (20.45%)	2 years P, F _{1-F3} generations	C = 0 VH = 55.8	Spent fuel rods; canned, shipped frozen; irradiated at 60 °F; stored at rt	C, VH	P 20M, 34F F ₁ 20M, 25F F ₂ 11M, 12F for C; 15M, 25F for VH F ₃ 6M, 6F	NHDIR/PEND. Only 2nd-litter animals used in F generation growth studies. Decreased weight gain in females of 4th generation. Increased cytochrome oxidase activity of male rats.	Read et al. (314)

Table 27 (continued)

Food type (% in diet) ^a	Study type/ duration	Irradiation dose (kGy) ^b	Process conditions	Group	Animals per group	Comments	Author/ reference
Mixed: beef (33% of wet weight), pork (10%), cheddar cheese (20%) and milk powder (12%)	2 years	C = 0 H = 27.9	Spent fuel rods; foods canned, shipped frozen; irradiated at 60 °F; stored at rt	C, H	20M, 20F	NHDIR. Sprague-Dawley. Mix of 5 foodstuffs fed 50:50 with synthetic basal diet.	Tepley & Kline (315)
Mixed: pork brain (5%) and egg (2.5%)	2 years	C = 0 EH = 93	Spent fuel rods; foods canned separately, shipped frozen; irradiated at 60 °F, stored at rt for 3–9 months	C, EH	20M, 20F	NHDIR. Sprague-Dawley. Brain and whole egg were irradiated separately, then dried.	Tepley & Kline (313)
Oil, soya (20%)	280 days	C = 0 2.2, 8.8, 44, 88 kGy	1 MeV electrons (van der Graaf); irradiated at 25 °C	C/0 T/1 T/4 T/20 T/40	40	NHDIR/PEND. No toxic effects at 2.2 kGy. Increased mortality at 8.8 kGy at 40 weeks; apparent after 24 weeks at 44 kGy. 88 kGy group had reduced growth and PER at 10 weeks, 18% mortality in 6 months. Mortality attributed to polymerization and autoxidative changes after extreme irradiation of oil.	Lang (344)
Oil: beef sterols (bs) and yeast sterols (y) (2.4% in oil; 0.8% in diet)	2 years, P, F ₁ , F ₂ generations	C = 0 H = 27.9 VH = 55.8	Electron radiation; frozen 1/4-inch slabs; GE Labs, Milwaukee, WI	9 groups in 3x3 matrix with (bs) and (y)	15M, 15F for C; 5M, 5F for all other groups	NHDIR/PEND. Sprague-Dawley. Study done in replicate. No carcinogenicity.	Tepley & Kline (313)
Oil: corn (3%), cottonseed (3%), peanut (3%)	2 years	C = 0 VH = 55.8	Electron radiation; mixture, refrigerated; GE Labs, Milwaukee, WI	C, VH	20M, 20F	NHDIR. Sprague-Dawley.	Tepley & Kline (315)

Table 27 (continued)

Food type (% in diet) ^a	Study type/ duration	Irradiation dose (kGy) ^b	Process conditions	Group	Animals per group	Comments	Author/ reference
Peach (35%)	2 years, P, F ₁ , F ₂ generations	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; canned in syrup, shipped frozen; irradiated (Dugway)	C, H, VH	25M, 25F	NHDIR. Wistar. No diet-related effects.	Tinsley, Bone & Bubl (327)
Pork (35%)	8-12 weeks	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; canned meats, shipped frozen; irradiated at 60 °F, stored at rt	C, H, VH	10M	NHDIR/PEND. Sprague-Dawley, males only. Increase in liver cytochrome oxidase in animals fed rt stored pork or beef, whether raw or cooked. (See also under beef.)	Read et al. (319)
Pork (35%): cooked prior to mixing in ration	2 years, P, F ₁ , F ₂ generations	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; ground pork canned under vacuum, frozen; irradiated at 60 °F, stored at rt	C, H, VH	6M, 6F	NHDIR. Wistar. No differences in growth, breeding and longevity.	Bubl & Butts (328); Bubl (329)
Pork (35%): pigs fed conventional diet = C+; pigs fed autoclaved diet = auto; pigs fed 50-kGy irradiated diet = irradi	2.5 years with F ₁ generation	C = 0 H = 37 VH = 74	Canned at 70 °C, pasteurized, stored at -40 °C; ⁶⁰ Co, -30 °C, stored at rt	CC+, C auto, auto, C irradi, H irradi VH irradi	P 12M, 24F F ₁ 50M, 50F	NHDR. SPF-derived Wistar, Riv:TOX. No effect attributable to irradiation. Tumour incidence and appearance comparable in all groups, none of any particular or unusual type.	van Logten (341)
Pork ground (p) (35%), bread (br) (80%), green beans (gb) (35% and 80%) and shrimp (sh) (35% and 80%)	84 days	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; (p), (gb), (sh) (cooked), canned, frozen; irradiated in pool; stored at rt for 3-8 months; (br) 0.25 and 0.5 kGy	C, H, VH	20M, 20F	NHDIR. Albino rats. Interest centred on blood enzymes. No effects related to diets or to amount of (gb) or (sh) in diet.	Brin, Ostashev & Kailinsky (330)

Table 27 (continued)

Food type (% in diet) ^a	Study type/duration	Irradiation dose (kGy) ^b	Process conditions	Group	Animals per group	Comments	Author/reference
Shrimp (35%); control shrimp received frozen, stored frozen; control and irradiated oranges, stored at 34 °F and 70% RH for 60–90 days	2 years, P ₁ , F ₁ –F ₃ generations	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; Canned cooked shrimp; irradiated at 60 °F, stored at rt Oranges: surface irradiated with electrons at Michigan State University, MI	C, H, VH	15M, 15F for C; 5M, 5F for all other groups	NHDIR. Sprague-Dawley. Oranges irradiated at 1.4 and 2.79 kGy included in diets of some groups.	Phillips, Newcomb & Shanklin (337)
Spice mixture (sm) (25%); pepper (pep) (3.5%); mild paprika (pap) (25%); feeding within 2 weeks of irradiation	15 days teratology	Chow = 0 C = 0 H = 15	⁶⁰ Co NS	Chow C H	pap/pep/sm 13/11/8 12/12/15 14/12/14	NHDIR. CFY albino female rats. No teratological effects in offspring of treatment groups. The slight increase in incidence of hydro-nephrosis for groups fed irradiated black pepper and paprika not considered related to the diet.	IFIP (337)
Strawberry (5%); juice (j) and powder (p); j, frozen, defrosted, homogenized 1:1 H ₂ O; p, frozen, lyophilized, powdered, stored N ₂ , canned	84 days	Chow C H	3 MeV electrons; fresh in PE bags; stored frozen	C, M, VH	10M, 10F	NHDIR/PEND. Wistar. Growth retardation in male groups consuming high-dose powder. No significant effects on females and all animals given high-dose juice.	Verschuren, van Esch & van Kooy (345)
Tuna (35%) and control tuna; received frozen, stored frozen	2 years P ₁ , F ₂ generations	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; canned; irradiated at 60 °F; stored at rt	3 control groups, 3x3 matrix	10M, 10F (11 groups) 6M, 6F for repro study for P, all groups	NHDIR. Charles-River; Wistar. Groups in 2-year study, no significant difference. Repro: no significant difference in reproduction among groups.	Paynter (332)

exp = experiment(s); F = female; F₁, F₂ = first filial generation, second filial generation, etc.; M = male; NHDIR = negative for high-dose irradiation effect; NS = not specified; P = parent generation; PE = polyethylene; PEND = possible effect of nutrition or diet; repro = reproduction study; RH = relative humidity; rt = room temperature.

^a Based on dry weight unless otherwise indicated.

^b Food irradiation dose levels: C = control; L = low (<10 kGy); H = high (≥ 10 kGy); VH = very high; EH = extremely high.

^c Texas Agricultural Experimental Station.

Table 28
Food type – mouse studies

Food type (% in diet) ^a	Study type/ duration	Irradiation dose (kGy) ^b	Process conditions	Group	Animals per group	Comments	Author/ reference
Bacon (35%)	750 days	Chow = 0 C = 0 H = 55.8	Spent fuel rods; canned, frozen; irradiated at 60 °F; stored at 5 °C for first 6 months, then at rt	Chow, C H	0 and 92 150 and 142 147 and 139	NHDIR. C3H and A/CrgI. Fat portion of bacon was separated and added back to laboratory diet. No major differences between experimental and control groups.	McKee et al. (346); Dixon et al. (347)
Chicken (35%): frozen (f), thermal (t), gamma (g), electron (e)	Reproduction and teratology 20 days	Chow = 0 C = 0 VH = 59	⁶⁰ Co, heated; canned, frozen; e, 10 MeV electrons, heated, vacuum packed in pouch, frozen	Chow, C, VH	60M, 60F for f, 40M, 40F for other groups	NHDIR. CD-1. Temporarily removed from the 730-day carcinogenicity study (for 25 weeks) to serve as P generation.	Raltech Scientific Services (356)
Chicken (35%): frozen (f), thermal (t), gamma (g), electron (e)	730 days	Chow = 0 C = 0 VH = 59	g, ⁶⁰ Co, heated; canned, frozen; e, 10 MeV electrons, heated, vacuum packed in pouch, frozen	Chow, C, VH	175M, 175F for f, 115M, 115F for all other groups	NHDIR. CD-1. Questionable incidence of interstitial cell tumours of the testes from (g) and (e) groups; after review of microslides FDA Cancer Assessment Committee (CFSAN) concluded no statistical or biological basis for tumour induction in testes of CD-1 mice.	Raltech Scientific Services (357)
Laboratory diet (100%)	60 days	C = 0 VH = 60	⁶⁰ Co NS	C, VH	30–40 M and F	NHDIR. Swiss. Compared haematology.	Maffei, Mazzali & DeSantis (358)
Laboratory diet (100%)	730 days P, F ₁ , F ₂ generations	C = 0 VH = 60	⁶⁰ Co 6 kGy/h	C, VH	P 9 F ₁ 15 F ₂ 20	NHIR/PEND. Swiss. Decrease in growth and fertility attributed to nutritional deficit (no vitamin supplements).	Biagini et al. (359)

Table 28 (continued)

Food type (% in diet) ^a	Study type/duration	Irradiation dose (kGy) ^b	Process conditions	Group	Animals per group	Comments	Author/reference
Laboratory diet (100%); autoclaved (auto), irradiated	Reproduction lifetime, 3 generations	C = 0 Auto = 0 VH = 44	⁶⁰ Co; paper cartons, plastic bags; auto at 120 °C, for 20 min	C, auto, VH	140M, 140F	NHDIR. Comparison of autoclaved and irradiated diet.	Saint-Lébe (336)
Laboratory diet (100%); diets 1 and 2, autoclaved (auto) or irradiated (I)	Reproduction and teratology 200 days	C = 0 H = 25	⁶⁰ Co; other conditions NS	1-auto 1-I 2-auto 2-I	12M, 24F for diet 1; 12M, 12F for diet 2	NHDIR. LACA and A2G. 2 strains, 4 diets = 8 groups. Comparison of autoclaving/irradiation; with diet 1, autoclaving resulted in higher number of litters; with diet 2, irradiation resulted in higher number of litters. Mice had a strong preference for the irradiated diet compared to the autoclaved diet.	Porter & Festing (360)
Mixed: beef (21.8%), tuna (14.3%), corn (30.8%), sweet potato (24.2%), fruit compote (8.9%), on wet weight basis	(12–28 months) 25 24 24 15 24 12 27 28 26 24	C = 0 VH = 55.8	Spent fuel rods; foods canned, frozen; irradiated at 60 °F; stored at rt	C, VH	10 replicates S3/C5 200/260/200 S3/C5 222/126/192 3/D 52/280 3 218 3 172 3 144 3 238 3 110 S 213 S 207	NHDIR. Swiss (S), C3H (3), C57 black (C5), DBA (D). Strains acquired from different sources. The strains used had a high incidence of certain tumours. No diet-related effects. Irradiated diet not carcinogenic in mice.	Deichmann (348); Radomski et al. (349)
Mixed: beef stew, codfish, chicken stew, green beans, peaches, and flour (16.67% each)	2 years	C = 0 VH = 55.8	Spent fuel rods; foods canned, frozen; irradiated at 60 °F; stored at rt	C, VH	Cal A strain: C 106M, 95F; VH 100M, 91F; C3H-NT strain: C 117M, 113F; VH 119M, 109F	NHDIR. Cal A and C3H-NT. Flour irradiated at 0.744 kGy; other components at 55.8 kGy. No significant differences between test and control mice of both strains for growth, mortality and reactions, and tumour incidence.	Calandra & Kay (350)

Table 28 (continued)

Food type (% in diet) ^a	Study type/ duration	Irradiation dose (kGy) ^b	Process conditions	Group	Animals per group	Comments	Author/ reference
Mixed: pork (8.8%), chicken (6%), evaporated milk (19.3%), potatoes (19.3%) and carrots (43%) on wet weight basis	19 months	Chow = 0 C = 0 VH = 55.8	Spent fuel rods; foods canned, frozen; irradiated at 60 °F; potatoes irradiated at 0.1 kGy, stored at 5–10 °C	Chow C, VH	200M, 200F	NHDIR/PEND. Strong A, Dba, Cb. Auricular dilatation with highest incidence in Cb strain. Additional testing suggests lesion due to mineral deficiency (copper).	Monsen (357–353)
Mixed: pork (8.8%), chicken (6%), evaporated milk (19.3%) and carrots (43%) on wet weight basis	600 days (II) 300 days (III) 600 days (IV) lifetime (V)	Chow = 0 C = 0 VH = 55.8	Spent fuel rods; foods canned, frozen; irradiated at 60 °F; potatoes irradiated at 0.1 kGy, stored at 5–10 °C	Chow C, VH	II-1200/strain, 3 groups; III-54M, 54F IVa-320, 5 groups; IVb-36M, 36F IVc-42M, 42F IVd-36M, 36F	NHDIR. Cb and Strong A (II), Cb for all others. Repeat of Monsen's study (II), to determine pathogenesis of heart lesions (III); etiology (IV); 100% milk diet (V). No "Monsen heart lesions" found.	Thompson et al. (354, 355)
Mixed: pork brain (5%) and whole egg (2.5%)	14 months	C = 0 EH = 93	Spent fuel rods; canned, separately, frozen; irradiated at 60 °F; stored at rt for 3–9 months	C, EH	48	NHDIR. S-P Swiss.	Tepley & Kline (375)
Oil: concentrate of beef and yeast sterols (2.4%)	15–22 months	C = 0 VH = 55.8	Electron radiation; frozen 1/4-inch slabs, GE Labs, Milwaukee, WI	C, VH	48-S-P Swiss 50-CAF1, JAX 60-C3H JAX	NHDIR. S-P Swiss; CAF1, JAX; C3H JAX. (Dissolved in oil component of diet before mixing.)	Tepley & Kline (373)
Oil: corn (3%), cottonseed (3%), peanut (3%)	15 months	C = 0 H = 27.9 VH = 55.8	Electron radiation; mixed, stored at refrig. temp., GE Labs, Milwaukee, WI	C, H, VH	50	NHDIR. S-P Swiss. (Mixed equal parts of non-irradiated oil and electron irradiated oil.)	Tepley & Kline (373)

Table 28 (continued)

Food type (% in diet) ^a	Study type/ duration	Irradiation dose (kGy) ^b	Process conditions	Group	Animals per group	Comments	Author/ reference
Wheat flour (50%)	Lifetime P, F ₁ , F ₂ generations	C = 0 VH = 50	⁶⁰ Co 1.8 kGy/h; stored at ambient temp	C, VH	P 2M, 10F F ₁ 10M, 50F	NHDIR/PEND. C57BL. Wheat flour fed within 1 week after irradiation. Effects on longevity, fertility presumed due to formation of peroxides and radicals (see reference 421). Reported loss of lipids and carotenoid fractions in irradiated diet.	Bugyaki et al. (361)

F = female; F₁, F₂, etc. = first filial generation, second filial generation, etc.; M = male; NHDIR = negative for high-dose irradiation effect; NS = not specified; P = parent generation; PEND = possible effect of nutrition or diet; repro = reproduction study; rt = room temperature.

^a Based on dry weight unless otherwise indicated.

^b Food irradiation dose levels: C = control; L = low (<10 kGy); H = high (≥ 10 kGy); VH = very high.

Table 29

Food type – dog studies

Food type (% in diet) ^a	Study type/ duration	Irradiation dose (kGy) ^b	Process conditions	Group	Animals per group	Comments	Author/ reference
Bacon (35%)	2 years	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; canned, frozen; irradiated at 60 °F in pool; stored at rt	C, H, VH	2M, 2F	NHDIR. No differences in growth, weight maintenance, haemoglobin, packed cell volume, white blood cell counts, reproduction and lactation.	Hale, Schroeder & Sikes (362)
Beef (35%)	25 weeks	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; canned ground beef; frozen; irradiated at 60 °F; stored at rt	C, H, VH	4M	NHDIR. Beagles. No significant differences in weight gains or blood values attributable to the diet.	Reber et al. (363)
Beef (35%)	104 weeks P generation, reproduction	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; canned ground beef; frozen; irradiated at 60 °F; stored at rt	C, H, VH	2M, 2F	NHDIR. Beagles. No diet-related effects.	Reber et al. (364)
Beef (35%)	Reproduction and feratology, 3 years	C = 0 VH = 60	11–12 MeV electrons; canned ground beef; frozen; irradiated at 60 °F; stored at rt	C, VH	3M, 15F	NHDIR. Beagles. No effect on growth rate, reproductive performance or general health.	Loosli et al. (375)
Beef (35%)	Reproduction, 104 weeks	C = 0 VH = 55.8	Spent fuel rods; canned ground beef; frozen; irradiated at 60 °F; stored at rt	C, VH	3M, 3F	NHDIR. Beagles. No adverse effect.	Clarkson & Pick (365)
Beef (35%)	2–3 years	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; raw beef, cellophane packaging; frozen; van de Graaf electrons	C, H, VH	6F	NHDIR. Beagles. No adverse effect.	McCay & Rumsey (371)

Table 29 (continued)

Food type (% in diet) ^a	Study type/ duration	Irradiation dose (kGy) ^b	Process conditions	Group	Animals per group	Comments	Author/ reference
Beef (35%)	2 years	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; canned, frozen; irradiated at 60 °F; stored at rt	C, H, VH	2M, 2F	NHDIR/PEND. Beagles. No differences in growth. (See also under chicken and pineapple jam.)	Blood et al. (367)
Beef stew (35%)	2 years	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; canned, frozen; (Dugway)	C, H, VH	2M, 2F	NHDIR. Beagles. No significant difference between groups.	Deichmann (369); Radomski et al. (326)
Cabbage (35%)	2 years	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; raw shredded, in plastic bags, in fibre drum, irradiated at 60 °F	C, H, VH	2M, 2F	NHDIR. No differences in growth, weight maintenance, haemoglobin, packed cell volume, white blood cell counts, reproduction or lactation.	Hale, Schroeder & Sikes (362)
Chicken (35%)	2 years	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; canned, frozen; irradiated at 60 °F; stored at rt	C, H, VH	2M, 2F	NHDIR/PEND. Beagles. No differences in growth. (See also under beef stew and pineapple jam.)	Blood et al. (367)
Chicken (35%): frozen (f), thermal (t), gamma (g), electron (e), or chow (c) 100%	Reproduction, F 36 months, M 40 months	C (c, f, t) = 0 H = 27.9 VH = 59	g = ⁶⁰ Co; heated, canned, frozen; e = 10 MeV electrons; heated, vacuum packed in pouch, frozen	C, VH	10M, 20F	NHDIR. Separate studies with gamma- and electron-irradiated chicken meat. No treatment-related effects.	Raltech Scientific Services (372)
Chicken stew (35%)	2-3 years	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; canned, frozen	C, H, VH	2M, 2F for C 2M, 3F for H, VH	NHDIR. Beagles. No adverse effect.	McCay & Rumsey (371)

Table 29 (continued)

Food type (% in diet) ^a	Study type/ duration	Irradiation dose (kGy) ^b	Process conditions	Group	Animals per group	Comments	Author/ reference
Chow (100%): Vetacan (meat feed mixture) (Vc) Vetavit (grain feed mixture) (Vv)	90 days	C = 0 H = 25	⁶⁰ Co	C, H	2 (4 groups)	NHDIR/PEND. 35% destruction of essential amino acids and lipid oxidation in Vc feed and carbohydrates in Vv feed. No noticeable sensory effects on feed, but test animals demonstrated drop in total proteins and creatine in blood serum. No other biological effects noted.	Smid, Dvorak & Hrusovsky (374)
Evaporated milk (35%)	2 years	C = 0 H = 27.9 VH = 55.8	Spent fuel rods, canned, frozen; (Dugway)	C, H, VH	2M, 2F	NHDIR. Beagles. No significant difference between groups.	Deichmann (366); Radomski et al. (326)
Fruit compote (35%)	104 weeks	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; canned; irradiated at 60 °F; stored at rt	C, H, VH	2M, 2F	NHDIR. Beagles. No diet-related effects on food consumption, growth measurements, feed efficiency, reproduction, haematology or histopathology.	Larson et al. (368)
Green beans (35%)	104 weeks	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; canned; irradiated at 60 °F; stored at rt	C, H, VH	2M, 2F	NHDIR. Beagles. No diet-related effects on food consumption, growth measurements, feed efficiency, reproduction, haematology or histopathology.	Larson et al. (368)

Table 29 (continued)

Food type (% in diet) ^a	Study type/ duration	Irradiation dose (kGy) ^b	Process conditions	Group	Animals per group	Comments	Author/ reference
Pineapple jam (35%)	2 years	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; canned, frozen; irradiated at 60 °F; stored at rt	C, H, VH	2M, 2F	NHDIR/PEND. Beagles. No differences in growth. Control group for jams was midway between H and VH groups. All dogs in "jam" groups had glycosuria. (See also under beef stew and chicken.)	Blood et al. (367)
Pork (35%)	4 years	NS	NS; fresh pork packed in plastic pouches	C, H	8M, 8F	NHDIR. Chow. No significant differences between groups for any parameter.	Cheng & Zhang (373)
Pork (35%)	90 weeks	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; canned, frozen; irradiated at 60 °F; stored at rt	C, H, VH	2M, 2F	NHDIR. Beagles. No deviations from normal.	McCay & Rumsey (369, 371)
Tuna (35%)	2 years	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; canned, frozen; irradiated at 60 °F; stored at rt	C, H, VH	2M, 2F for C 2M, 3F for H, VH	NHDIR. Beagles. No deviations from normal.	McCay & Rumsey (370, 371)

F = female; M = male; NHDIR = negative for high-dose irradiation effect; NS = not specified; PEND = possible effect of nutrition or diet; rt = room temperature.

^a Based on dry weight unless otherwise indicated.

^b Food irradiation dose levels: C = control; L = low (<10 kGy); H = high (≥ 10 kGy); VH = very high.

Table 30

Food type – miscellaneous animal studies

Food type (% in diet) ^a	Test species/ study duration	Irradiation dose (kGy) ^b	Process conditions	Group	Animals per group	Comments	Author/ reference
Barley meal (50%)	Japanese quail, 26 days	C = 0 H = 10 VH = 1000	10 MeV electrons, 250 mA, 0.9 cm/s at 40–50 °C	C, H, VH = C ⁺	30	NHDIR/PEND Reported decreased lymphocytes, leukocytes, blood serum triglycerides. Author's suggested need for further studies to determine the responsible agent in irradiated barley and whether it leads to a toxic effect.	Koch et al. (377); Döllstädt et al. (378)
Chicken (35% or 70% of diet), frozen (f), thermal (t), gamma (g), electron (e), or chow (c) 100%	Hamsters, reproduction and teratology, 5 days	C (c, f, t) = 0 VH = 59	g = ⁶⁰ Co; heated, canned, frozen; e = 10 MeV electrons; heated, vacuum packed in pouch, frozen	C, VH	5	NHDIR. No teratogenic response in groups receiving irradiated diet.	Raltech Scientific Services (379)
Feed (100%): autoclaved for 10 min at 120 °C (auto); irradiated	Pigs, 16 weeks; 90 days, 3-generation reproduction	C = 0 Auto = 0 VH = 50	⁶⁰ Co; other conditions NS	C, auto, VH	4M, 12F	NHDIR. Phase I of a relay study. No treatment- related effects.	Strik (380)
Laboratory diet (100%) with 10% soy bean oil	Chicks, 5 weeks	VH = 30 VH = 60	300 KCl; ⁶⁰ Co; soybean oil in glass bottle. 100 KCl; ⁶⁰ Co; whole diets in cardboard box	Exp I, 5 groups; Exp II, 9 groups	5	NHDIR/PEND. Growth depression and effects attributed to autooxidation of lipids in irradiated diet.	Tagigawa, Danbara & Ohyama (381)
Peaches (35%)	Rhesus monkeys, 24 months	C = 0 H = 27.9 VH = 55.8	Spent fuel rods; canned in syrup	C, H, VH	2M, 2F	NHDIR/PEND. Experiment also included animals fed whole and peeled oranges irradiated to 1.5 and 3 kGy with electron source. No diet-related effects.	Blood et al. (376)

Exp = experiment; F = female; M = male; NHDIR = negative for high-dose irradiation effect; NS = not specified; PEND = possible effect of nutrition or diet.

^a Based on dry weight unless otherwise indicated.^b Food irradiation dose levels: C = control; L = low (<10 kGy); H = high (>10 kGy); VH = very high or EH = extremely high.

Table 31
Food type – mutagenicity studies in vitro

Food type	Species type/ duration	Irradiation dose (kGy)	Process conditions	Comments	Author/reference
Beef, pork, veal	<i>Salmonella</i>	50	10 MeV electrons NS	NHDIR. All fried meat samples mutagenic. Irradiation not a factor.	Münzer (394)
Cod	<i>Salmonella</i>	12	⁶⁰ Co at 4 °C; storage at rt 1, 2, 7, 14, 21 days in plastic film bags	NHDIR. No mutagenic effects.	Joner, Underdal & Lunde (382)
2-Dodecylcyclo- butanone (2-DCB) (radiolysis product from palmitic acid moiety in triglyceride)	Rat and human colon cells	Not applicable	Synthesized	PEHDIR. 2-DCB at 0.3–1.25 mg/ml induces DNA strand breaks in rat and human colon cells. Concentrations tested were high compared with actual human intake. <i>In vivo</i> test in progress in rats. Chicken meat from Raltech study contained 2-DCB at only 17 µg/g lipid.	Delincée & Pool-Zobel (427)
Glucose, peptone	<i>Escherichia coli</i>	50	6.5 kCi ⁶⁰ Co	NHDIR. No induction of lysogenic bacteria.	Bugyaki, Lafontaine & Moutschen- Dahmen (384)
Growth medium	Human cells	10 20	⁶⁰ Co 4.5 kGy/h; powder form in individual containers at rt; packed in plastic containers, dia. 13 cm	NHDIR. SCE frequencies did not show significant difference.	Vijayalaxmi (385)
Herring	<i>Salmonella</i>	12	⁶⁰ Co at 4 °C; storage at rt 1, 7, 14 days in plastic film bags	NHDIR/PEND. Saline and ethanol extracts tested in 6 <i>Salmonella</i> strains. Saline extracts not mutagenic. Two strains had positive response (twice background) when ethanol extract was concentrated two-fold.	Joner & Underdal (386)
Onion powder	<i>Salmonella</i>	13.6	⁶⁰ Co ambient, in unsealed plastic pouches in metal cans	NHDIR. No mutagenic effects.	Münzer & Renner (387)

Table 31 (continued)

Food type	Species type/ duration	Irradiation dose (kGy)	Process conditions	Comments	Author/reference
Paprika	<i>Salmonella</i>	50	⁶⁰ Co, aerobic, ambient	NHDIR. No mutagenic effects.	Central Food Research Institute (388)
Spice mix	<i>Salmonella</i>	15 45	⁶⁰ Co, aerobic, ambient	NHDIR. No mutagenic effects.	Farkas, Andrásy & Incze (389)
Strawberry	<i>Salmonella</i> Human	15	⁶⁰ Co, 3.6 kGy/h, 400-ml beaker, 25 °C	NHDIR. No significant differences using distillates or residue.	Schubert et al. (390)
Sucrose solution: concentrated to 20% for shipment to laboratory. Stored at rt or 5 °C for several months, diluted with saline before use	Human	20	⁶⁰ Co, 2% solution, concentrated to 20%	PEHDIR. Chromosomal breaks in human lymphocytes in cell culture. Attributable to radiation- induced chemistry of simple carbohydrate solutions (see reference 383). Concentration may have increased concentration of oxidized products.	Shaw & Hayes (391)
Sucrose solution	<i>Vicia faba</i>	20	⁶⁰ Co, 3.3 kGy/h; solution, NS	PEHDIR. Chromosome changes attributable to radiation-induced chemistry of simple carbohydrate solutions (see reference 383).	Bradley, Hall & Trebikock (392)
Sucrose, fructose, glucose, maltose solutions and model mango	<i>Salmonella</i>	50	⁶⁰ Co, 17 kGy/h; sealed ampoules, O ₂ enriched, 25 °C	PEHDIR. Irradiated simple sugar solutions mutagenic in one of five strains tested. Complex sugar model mango system not mutagenic.	Niermand et al. (383)
Sucrose, ribose solutions	<i>Salmonella</i>	20	⁶⁰ Co, 4.5 kGy/h; air, N ₂ 10 min, seal; dry ice; stored 6–8 °C for up to 25 weeks	PEHDIR. Mutagenic effect attributable to radiation- induced chemistry of simple carbohydrate solutions (see reference 383).	Aiyar & Rao (393)

NHDIR = negative for high-dose irradiation effect; NS = not specified; PEHDIR = possible effect of high-dose irradiation; PEND = possible effect of nutrition or diet; rt = room temperature.

Table 32
Food type – mutagenicity studies in vivo

Food type (% in diet) ^a	Species type/duration	Irradiation dose (kGy)	Process conditions	Comments	Author/reference
Beef, ham	<i>Drosophila</i>	59 beef, 39 ham	Beef, 3 MCi ⁶⁰ Co, canned, frozen; Ham, 10 MeV electrons, vacuum-packed, frozen	NHDIR. No mutagenic effects. No significant increases in recessive sex-linked lethals, loss of chromosomes or non-disjunction.	Mittler (395)
Black beans	Mouse Swiss-55 (10M)	15, 20	¹³⁷ Cs, 4 kGy/h; rt, 5 doses, stored for 2 weeks, boiled	NHDIR. Dominant lethal test. No differences in pregnancy rates, total implants, live and dead implants, sex distribution, or abnormalities.	Bernardes et al. (414)
Chicken (35%); chow (c only); frozen (f), stored at 0 °C; thermal (t), stored at 23 °C; gamma (g), stored at 23 °C; electron (e), stored at 23 °C	Mouse (c, 21M; f, 36M; t, 18M; g, 27M; e, 26M)	59	g = ⁶⁰ Co, heated canned, frozen; e = 10 MeV electrons, heated, vacuum-packed in pouch, frozen	NHDIR. Dominant lethal test. Feeding of radiation-sterilized chicken meat did not induce dominant lethal events. Positive control produced negative results, unsuitable for supporting safety.	Raltech Scientific Services (397)
Chicken (5, 25, 37.5 or 50% of diet); frozen (f); thermal (t); gamma (g); electron (e)	<i>Drosophila</i> (C-, C+, f, t, g, e)	55.8	g = ⁶⁰ Co, heated canned, frozen; e = 10 MeV electrons, heated, vacuum-packed in pouch, frozen	NHDIR. No mutagenic effects. Study noted decreased numbers of offspring in groups raised on irradiated chicken meat. FDA found no evidence of adverse reproductive effects.	Lusskin (396)
Glucose powder	Mouse Swiss	20, 50	⁶⁰ Co, 57 Gy/min; polyethylene bags, air, 25 °C	NHDIR. Dominant lethal test. No mutagenic effects.	Varma et al. (398)
Glucose powder	<i>Drosophila</i>	20, 50	⁶⁰ Co, 57 Gy/min; polyethylene bags, air, 25 °C	NHDIR. No mutagenic effects.	Varma et al. (399)
Glucose powder	Mouse Swiss (6 per group)	20, 50	⁶⁰ Co, 57 Gy/min; polyethylene bags, air, 25 °C	NHDIR. Host-mediated assay. No mutagenic effects.	Varma et al. (400)

Table 32 (continued)

Food type (% in diet) ^a	Species type/ duration	Irradiation dose (kGy)	Process conditions	Comments	Author/reference
Glucose powder	Mouse Swiss	20, 50	⁶⁰ Co, 57 Gy/min; polyethylene bags, air, 25 °C	NHDIR. Micronucleus test in bone marrow cells and chromosomal aberration assay. No evidence of mutagenic effects in somatic or germ cells.	Varma et al. (401)
Laboratory diet: Solid cakes	Mouse C57BL	50	⁶⁰ Co: mixed 1:1 with unirradiated food and pressed into new cakes prior to use	NHDIR/PEND. Dominant lethal test. Increased pre- implantation embryonic deaths; not confirmed by cytological analysis.	Moutschen-Dahmen, Moutschen & Ehrenberg (402)
Laboratory diet: Pellets, enriched with amino acids and vitamins	Rat SPF Wistar 10–20M, 30–60F	50	C = auto 110 °C, 10 min; Auto = 120 °C, 15 min; H = 10 MeV electrons	NHDIR. Dominant lethal test. No evidence of mutation.	Eriksen & Emborg (415)
Laboratory diet: Food pellets	Mouse Swiss (SPF) (10 per group)	0, 7.5, 15, 30	⁶⁰ Co, 8.5 kGy/h; NS	NHDIR/PEND. Host-mediated assay. Significant increase in the mutation frequency induced by the high dose irradiated food (see reference 407).	Johnston-Arthur et al. (403)
Laboratory diet 10% moisture	Rat Wistar (15M)	25	⁶⁰ Co, 2.4 kGy/h; NS; stored for 3–4 weeks at 4–6 °C	NHDIR. Dominant lethal test. No evidence of mutagenic effects.	Chauhan et al. (404)
Laboratory diet 10% moisture	Mouse Swiss	25	⁶⁰ Co, 2.4 kGy/h; NS	NHDIR. Dominant lethal test. No effect on post- implantation loss, dead implantations, or pregnancy.	Chauhan et al. (405)
Laboratory diet: pellets	Mouse	45	10 MeV electrons; NS; 10 min	NHDIR. Host-mediated assay. No mutagenic effects.	Münzer & Renner (416)
Laboratory diet	Mouse BALB/c	28.5	⁶⁰ Co, 11–11.5 h of exposure; 37–38 °C	NHDIR. Bone marrow and male germ cells examined for chromosome aberrations. No mutagenic effects.	Leonard, Wilcox & Schietcatte (406)

Table 32 (continued)

Food type (% in diet) ^a	Species type/duration	Irradiation dose (kGy)	Process conditions	Comments	Author/reference
Laboratory diet: pellets	Chinese hamster	45	10 MeV electrons; open aluminium trays	NHDIR/PEND. No increase in chromosomal aberrations; slightly increased incidence of polyploidy.	Renner (417)
Laboratory diet: pellets	Mouse (25-100 per group)	0, 7.5, 15, 30	⁶⁰ Co; 250 g pellets in polyethylene sacks; 22-24 °C	NHDIR/PEND. Host-mediated assay for 3 commercial food pellets. Irradiation increased mutation frequency between 10 and 60 fold for the 3 products compared to controls. Subsequent extraction study found mutagenic agent extracted by alcohol. Water extract had a lower effect and ether extract had no effect.	Johnson-Arthur et al. (407)
Laboratory diet	Mouse CD1	10, 25, 50	⁶⁰ Co	NHDIR/PEND. Dominant lethal test. Used 4 diets on 2 strains. Some evidence of weakly mutagenic effect with one diet.	Anderson et al. (408)
Laboratory feed	Mouse, SPF Ha/ICR (Swiss)	30	10 MeV electrons; NS	NHDIR. Host-mediated assay. No mutagenic effects.	Münzer & Renner (418)
Medium	<i>Drosophila</i>	30	25 MeV electrons (600 mA) on tungsten converter, 10 kGy/min; medium in lucite vials, used immediately or after 3 weeks	NHDIR/PEND. Offspring tested for dominant, sex-linked, and F ₃ lethality. No decrease in F ₁ survival or increase in F ₃ sex-linked lethality. There was small but consistent increase in sex-linked recessive lethality, but no detectable dose effect. (Low-dose exposures were with X-rays operated at 250 kV _p and 15 mA, 50 Gy/min.)	Rinehart & Ratty (419)
Medium, DNA	<i>Drosophila</i>	10	14 kCi ⁶⁰ Co; 70-min exposure	NHDIR. No evidence that irradiated medium or DNA is mutagenic.	Chopra (409)

Table 32 (continued)

Food type (% in diet) ^a	Species type/duration	Irradiation dose (kGy)	Process conditions	Comments	Author/reference
Medium, sucrose (30%), DNA	<i>Drosophila</i>	30	25 MeV electrons (600 mA) on tungsten converter, 10 kGy/min; sucrose solution in lucite vial, used immediately or after 3 weeks; diluted to 10% with medium	NHDIR/PEND. Autoclaved sucrose or DNA medium is mutagenic (irradiated or not). Non-autoclaved sucrose is not mutagenic. (DNA irradiated at low doses with X-rays operated at 250 kV _p and 15 mA, 50 Gy/min.)	Rinehart & Ratty (420)
Milk powder (35%)	Mouse, NMRI/Han (750 mice) Rat, Sprague-Dawley (716 rats), P-F ₅	45	10 MeV electrons, 10 min	NHDIR. Dominant lethal test, reproduction. High content of radicals in the irradiated food. No harmful effects.	Renner et al. (421)
Onion powder (10%)	Chinese hamster Mouse	13.6	⁶⁰ Co; unsealed plastic pouches in metal cans; ambient	NHDIR. Sister chromatid exchange tests negative in hamsters and 3 strains of mice.	Münzer & Renner (387)
Paprika	Mouse	50	⁶⁰ Co; aerobic, ambient	NHDIR. Host-mediated assay. No increase in number of revertants.	Central Food Research Institute (388)
Paprika (20%) (8.6% moisture)	Mouse Swiss	30	⁶⁰ Co; 3.1 kGy/h; NS; fed 8–18 days after irradiation	NHDIR. Micronucleus test. No differences in the incidence of erythrocytes with micronuclei, and polychromatic:normal ratio comparable among all groups.	Chaubey et al. (408)
Spice mix Pepper	Rat CFY	15	⁶⁰ Co; aerobic, ambient	NHDIR. <i>E. coli</i> induction on blood of rats. No induction of lysogenic bacteria.	Farkas & Andrassy (411)
Spice mix	Rat CFY	15, 45	⁶⁰ Co; aerobic, ambient	NHDIR. Negative Ames test on irradiated spice extracts and on urine of rats fed irradiated spices.	Farkas, Andrassy & Incze (389)

Table 32 (continued)

Food type (% in diet) ^a	Species type/ duration	Irradiation dose (kGy)	Process conditions	Comments	Author/reference
Spice mix (25%)	Rat Sprague-Dawley	15	⁶⁰ Co, 0.5 kGy/h; NS; feeding 5–9 days after irradiation	NHDIR. Dominant lethal test. No significant difference between irradiated spice groups and controls.	Barna (412)
Strawberry	Mouse	15	⁶⁰ Co; 25 °C	NHDIR. No clastogenic effects.	Schubert et al. (390)
Sucrose, ribose solutions	Mouse	50	⁶⁰ Co, 4.5 kGy/h; air, N ₂ 10 min, seal; dry ice; stored at 6–8 °C up to 25 weeks	NHDIR. Host-mediated assay. No increase in number of revertants.	Aiyar & Rao (393)
Wheat (50%)	Mouse (2M, 10F per group in P, 10M, 50F per group in F ₁)	0, 50	3.1 kCi ⁶⁰ Co, 1.8 kGy/h; plastic bags	NHDIR/PEND. Chromosomal abnormalities in germ cells presumed due to formation of peroxides and radicals (see reference 421) with subsequent loss of lipids and carotenoid fractions in irradiated diet.	Bugyaki et al. (361)
Wheat (freshly irradiated)	Chinese hamster, 72 h after feeding; Rat, 12 weeks	0, 15, 30	⁶⁰ Co; N ₂ , air	NHDIR. No difference in polyploids in bone marrow cells or micronuclei in reticulocytes 72 h after diets irradiated in N ₂ or air. Analyses of micronuclei in peripheral blood of rat fed wheat flour irradiated at 0.75 kGy done at 6 and 12 weeks.	Tanaka et al. (413)

F = female; M = male; NHDIR = negative for high-dose irradiation effect; NS = not specified; PEND = possible effect of nutrition or diet; rt = room temperature.

^a Based on dry weight unless otherwise indicated.

The United States Army became interested in the feasibility of preserving foods by ionizing radiation from radioisotopes and radioactive by-products from nuclear reactors in the early 1950s (423). As part of the Army's overall programme, the Medical Research Branch of the Surgeon General's Office was assigned the task of determining the wholesomeness of radiation-sterilized foods in 1953. This programme supported studies in academic and research institutions as well as in military research institutions, and resulted in many of the feeding studies undertaken in rats, mice, dogs and monkeys. There were initially 49 foods under investigation in short-term studies; 21 were chosen for long-term toxicity studies, including ground beef, pork loin, bacon, shrimp, cod, chicken, tuna, beef stew, chicken stew, carrots, cole-slaw, corn, green beans, potatoes, sweet potatoes, flour, fruit compote, evaporated milk, peaches, oranges and jam.

The high doses used and the quantity of food tested in the animals were greater than those that would normally be encountered and consumed, in order to maximize any potential toxicity. The large group of foods used for the studies reflected the concern at that time that each food item, or a combination of irradiated foods, might respond to irradiation in a unique way (314, 320). The research also tried to correct the problem of palatability of diets containing high levels of irradiated food items by taking caloric consumption into account in the statistical evaluation of the results (320).

The 1994 WHO publication on the safety and nutritional aspects of irradiated food included a section evaluating the studies in the United States Food and Drug Administration (FDA) electronic database (10). Following the 1981 Bureau of Foods Irradiated Food Committee Report, in which it was concluded that, on the basis of studies on the radiation chemistry of foods, an adequate margin of safety can be demonstrated for foods irradiated below 10 kGy and for dry and dehydrated spices that are irradiation sterilized, the FDA reviewed all available animal studies to determine their adequacy and to evaluate the toxicological evidence (424). This review of over 400 studies resulted in over 250 being "accepted" or "accepted with reservation", and about 150 being "rejected"; some 20 review articles were not categorized. On the basis of this additional review and evaluation, the FDA confirmed its earlier conclusions regarding the safety of foods permitted by regulation in 1986.

The FDA stated that five of the studies reviewed were considered to have been properly conducted, fully adequate by 1980 standards, and capable of standing alone to support the safety of irradiated foods (424). Of those five, two were with foods irradiated to a dose greater than 10 kGy: dried

milk to 45 kGy (343) and beef stew and evaporated milk to 27.9 and 55.8 kGy, respectively (326).

The Study Group's evaluation of the safety of foods irradiated at doses greater than 10 kGy included some of the studies "rejected" by the FDA reviewers. The studies had been rejected for one or more reasons: the radiation dose was not reported; the number of animals per group was not reported; the number of animals per group was small (less than five); the study was conducted without controls fed a non-irradiated diet; the diet fed was determined to be nutritionally inadequate; and the studies were conducted at a laboratory that was considered by the FDA to be in violation of good laboratory practice (424). Nevertheless, their inclusion in the present evaluation provides a broader perspective on the diverse data obtained.

6.3.1 ***Subchronic studies***

Many subchronic studies on safety have been conducted in rats (12), mice (1), dogs (2), pigs (1), quails (1) and chickens (1) (Tables 23–26). These studies examined the safety and nutritional adequacy of a variety of dietary items and complete laboratory diets treated with high-dose irradiation. The vast majority of these studies reported no toxic effects in laboratory animals after consumption of high-dose irradiated foods.

The few adverse events in these studies appeared to reflect degradation of essential nutrients in treated diets. In 1963, Malhotra et al. reported that high-dose irradiated beef (55.8 kGy) fed to rats at 35% of the diet resulted in excess mortality from a haemorrhagic syndrome in males that could be prevented by dietary supplementation with vitamin K (315–317). They also reported that administration of testosterone increased mortality linearly and that the effect of methionine was protective and decreased mortality linearly; these factors were independent of each other. Other detailed investigations of similar adverse findings in subchronic toxicity studies ultimately demonstrated that they were attributable either to preexisting nutritional deficiencies in the diets or to nutrient degradation not unique to irradiation (339, 344, 345, 374, 381).

6.3.2 ***Carcinogenicity and chronic toxicity studies***

Several studies on high-dose irradiated diets were conducted using rodents, primarily rats, and following protocols that involved two-year carcinogenicity bioassays and multigeneration reproductive toxicology evaluations. There were 17 such combined studies in rats (Table 23), three in mice (Table 24), and one in pigs (Table 26). Some of the studies were conducted with radiation-sterilized laboratory diets.

Additional carcinogenicity bioassays without reproductive components have been reported for rats and mice (Tables 23 and 24). This large collection of carcinogenicity data is unique in the assessment of all food-related treatments and processes. No irradiation-related increases in tumours occurred in any of the studies that involved administering high-dose irradiated foods or diets to rats or mice. Similarly, no irradiation-induced changes in reproductive function were reported in the multi-generation reproduction phases of the combined carcinogenicity-reproduction studies.

Chronic toxicity studies have been conducted in mice (4), dogs (7) and monkeys (1) (Tables 24–26). In one, an unusual heart lesion (auricular dilatation) was reported in a single mouse strain. This study involved three strains of mice fed three diets: a non-irradiated chow; a synthetic diet constituted from high-dose irradiated components; and a non-irradiated synthetic diet (351, 352). Monsen (351) reported auricular dilatation in mice fed a composite diet of irradiated pork, chicken, evaporated milk, potatoes and carrots. Thompson et al. at the Medical Research Laboratory tried to repeat Monsen's study and initiated additional tests to determine the pathogenesis of the heart lesions. However, they could not duplicate the effect (354, 355). Monsen conducted additional studies (352, 353) and reported that the effects were due to deficiency of iron and copper in the diet (353). The other chronic studies in mice did not show any adverse effects due to the high-dose irradiated diet or to the high-dose irradiated dietary components.

Chronic studies in dogs, conducted for durations of 2–4 years (Table 25), reported no adverse findings attributable to high-dose irradiated food (Table 29). Blood et al. (367) reported that dogs fed an irradiated chicken or beef diet showed no differences in growth compared to controls, but dogs fed an irradiated pineapple jam diet showed some differences and all dogs developed glycosuria as a result of the high-carbohydrate content. The authors noted four cases of primary lymphocytic thyroiditis, two in animals receiving chicken meat, one on the beef diet, and one on the jam diet (367). A review of the evidence of the presence of lesions in various organ tissues representing 273 dogs from all studies (326, 362, 364–366, 368–371) was made by the United States Armed Forces Institute of Pathology (425); thyroiditis in dogs was found to be a nonspecific lesion that had been reported to occur with equal frequency in irradiated and non-irradiated dietary groups. The duration of these dog studies was not adequate to assess carcinogenicity; nonetheless, there were no suggestions of pathological abnormalities in any chronic study conducted with dogs.

In a non-human primate study in which high-dose irradiated peaches (27.9 and 55.8 kGy) were fed to rhesus monkeys for a duration of two years, there were no adverse findings in male monkeys, but female monkeys demonstrated marked variations in acceptance of the semi-liquid irradiated diets. Untoward findings in female monkeys were attributed by the authors to problems consistent with decreased palatability of the diet and consequent rejection of the food (376).

The United States Office of the Surgeon General initiated a series of nutritional and toxicological studies on chicken meat sterilized by ionizing radiation in 1976 that was completed in 1984 (9). Most of these studies were conducted by Raltech Scientific Services (Raltech) of St Louis, Missouri, with specific portions assigned to other institutions. Responsibility for supervision of the Raltech contract was transferred from the Army to the United States Department of Agriculture in October 1980. The studies included a chronic feeding study in mice (357) and dogs (372). The chicken meat (deboned, 18% skin and 82% meat) was vacuum packed in cans or retort pouches (26 mm thick), thermally processed at 73–80 °C to inactivate the enzymes, cooled to –40 °C, and irradiated in the frozen state in the absence of air to a minimum dose of 45 kGy and to an average dose of 59 kGy. Samples irradiated by electron accelerator were sterilized by exposure to 10 MeV electrons at –25 °C. Control samples were kept frozen, and thermally treated samples were processed to an internal temperature of 115.6 °C to a sterility level of $F_0 = 6$ (9)¹.

As part of the FDA review, scientists from FDA and the National Toxicology Program's Board of Scientific Counselors reviewed the data and agreed that the evidence did not show any treatment-related induction of testicular tumours (424, 426).

On the basis of the above studies, the FDA concluded that there were no treatment-related effects in the mouse and dog feeding studies (424, 426).

6.3.3 **Reproduction and teratology studies**

The Netherlands National Institute of Public Health and Environmental Hygiene conducted a series of studies to determine the potential formation of toxic compounds in irradiated foods (340, 341, 380). In the first study, there were no observable differences between rats fed an irradiated diet (50 kGy) or an autoclaved diet (15 min at 120 °C) and those fed a control diet with respect to growth, feed consumption,

¹ Sterility level F_0 provides a basis for comparing the sterility level achieved by heat treatment at any temperature to that achieved by an equivalent treatment at 121 °C in terms of minutes. $F_0 = 6$ signifies that the heating time at 115.6 °C was sufficient to produce a sterility level equivalent to that achieved by heating at 121 °C for 6 minutes.

reproduction, haematology, urinary and organ histopathology parameters (340, 380). The second study was performed with pigs and involved three generations, two litters of piglets per generation, F_a and F_b (380). The F_a generation was used for continued breeding and the F_b generation was observed for gross abnormalities and was discarded at weaning age. There were no deviations in feed consumption, growth, haematological, and biochemical parameters in the animals, and the authors concluded that there were no treatment-related effects in the growth and reproduction of pigs fed irradiated or autoclaved feed for three generations. In the third phase of the study, three groups of 15 male and 15 female pigs from the F_{1a} generation fed 50 kGy-irradiated, autoclaved or control feed were slaughtered and processed to ham products (341, 380). Six groups each of 50 male and 50 female rats were then fed the following diets: (1) standard diet; (2) 35% ham from control pigs, treated with nitrite at 200 mg/kg; (3) 35% ham from pigs fed the autoclaved diet, treated with nitrite at 200 mg/kg and autoclaved; (4) 35% ham from control pigs, treated with nitrite at 50 mg/kg and irradiated to 37 kGy; (5) 35% ham from pigs fed the 50 kGy-irradiated diet, treated with nitrite at 50 mg/kg and irradiated to 37 kGy; and (6) 35% ham from pigs fed the 50 kGy-irradiated diet, treated with nitrite at 50 mg/kg and irradiated to 74 kGy. The authors concluded that there were no treatment-related effects in: feed consumption, growth, mortality, haematology, biochemistry of blood and urine, organ weights, histopathology and tumour incidence. In addition, the concentrations of nitrosamines in the ham did not change with added nitrite or irradiation dose.

Read et al. (314) at the United States Army Medical Research Laboratory in Denver, Colorado, also conducted long-term toxicity studies in rats fed a composite food diet, each irradiated to 55.8 kGy. They reported decreased weight gain in females of the F_3 generation, but urged caution in interpreting the results because of the small number of animals used. They concluded that the variations in reproductive performance did not indicate toxicity, but should be monitored in feeding trials. In addition, they reported increased cytochrome oxidase activity in this study and in an earlier study where rats were fed diets containing 35% beef or pork (319). The authors noted that cytochrome oxidase activity was not affected in diets with fruits and vegetables and suggested that the probable cause of the increase was the meat components present in the meat and composite diets, i.e. nutritional components rather than irradiation. A review of the evidence of lesions in various organ tissues representing over 3000 rats did not indicate any gross or histopathological lesions that could be specifically attributed to the irradiated diet (425). In a few organs, the differences occurred in both

test and control animals. Because several strains of rats were used, a comprehensive pooled data report was not compiled (A. Brynjolfsson, personal communication).

Several multigeneration reproduction studies were conducted in rats (1), mice (3), dogs (4), and hamsters (1) (Tables 23–26). Minor effects noted in some cases, generally involving small decreases in body weight or body weight gain in the later generations of multigeneration studies, appear to have been related to nutrition and reduced palatability of the diet. Reproductive and teratological end-points demonstrated no effects with any consistent pattern or trend.

6.4 Mutagenicity studies

Data from both *in vitro* and *in vivo* mutagenicity studies are presented in Tables 31 and 32, even though the emphasis in this report has been on high-dose irradiated foods tested directly in animal feeding studies. A few of these *in vitro* studies, but none of the *in vivo* studies, have shown mutagenic effects of certain irradiated substrates. However, the *in vitro* studies are of less relevance, since such data are not as valid as those from animal studies for the purpose of estimating risk to humans on the basis of extrapolation.

In this regard, the possible mutagenic activity of 2-dodecylcyclobutane (2-DCB), formed radiolytically from food containing fat, has received particular attention. A recent study employing single-cell gel electrophoresis (comet assay) indicated that 2-DCB in the concentration range 0.30–1.25 mg/ml produces some cytotoxicity and an associated but weak effect in DNA at alkali-labile sites (427). However, the concentrations used were far greater (about three orders of magnitude) than the 17 µg/g reportedly present in the extracted lipid of chicken meat irradiated to 59 kGy. It should also be noted that the concentration of 2-DCB actually present in high-dose irradiated chicken meat, when calculated on the basis of the total meat content, would be even smaller.¹

In contrast, studies in *Drosophila* (396) and mice (397) did not show any mutagenic activity of high-dose irradiated chicken (55.8 kGy and 59 kGy, respectively).

Similarly, Tanaka et al. (413) reported no difference from controls in polyploids in bone marrow cells or reticulocytes of Chinese hamsters fed wheat irradiated to doses of 0, 15 and 30 kGy.

¹ Note added in proof by the Secretariat: In a subsequent *in vivo* study, as yet unpublished, the researchers claim to have found a small positive effect when six rats were administered an extremely high level of the synthetically prepared 2-DCB. Limitations of the experiment, particularly the exclusive reliance on the unvalidated comet assay technique, call into question the significance of this finding. Another unpublished study found that the Ames test for 2-DCB was negative.

6.5 Human clinical studies

In a series of studies involving young male human volunteers, the United States Army evaluated the wholesomeness of foods treated with high-dose radiation. Subjects consumed irradiated foods for periods of 15 days separated by control and washout intervals. Generally, the experimental protocol called for a variety of foods (54 items) to be sealed in cans, frozen and irradiated to 25–40 kGy using gamma-rays from spent fuel rods in Dugway, Utah, and Arco, Idaho, then thawed and stored at room temperature. Non-irradiated control items were processed and stored similarly, unless freezing was required to avoid spoilage. Irradiated foods were tested for sterility and the presence of bacterial exotoxins prior to human consumption. Individuals and groups served as their own controls during the series of experimental periods.

Controlled housing in a metabolic ward was provided during testing and subsequent follow-up evaluations. Particular attention was paid to clinical examinations, cardiac performance, and haematological, hepatic and renal functions.

The first study involved 18 human volunteers. Half the subjects received a diet containing irradiated foods during the initial 15-day experimental period; the remainder received non-irradiated control items. The experimental conditions for the two groups were reversed during subsequent 15-day periods, separated by 5-day washout intervals. The proportion of calories from irradiated food in the experimental diets was increased sequentially from 35%, to 60%, to 80%, and finally to essentially 100% of metabolizable energy by the end of the study (429). No toxic effects were observed for any experimental diet, regardless of the proportion of high-dose irradiated food. No clinical changes were detected in any individual from baseline to post-exposure evaluations, or at follow-up examinations up to one year post-exposure.

In a second study, 10 human volunteers consumed a diet in which 32% of calories were derived from irradiated canned pork treated to 30 kGy. The irradiated canned pork was stored at room temperature for one year prior to consumption; control pork was fresh and obtained locally. The irradiated diet contained no vitamin K supplementation (310). The study design consisted of two 15-day exposure periods in which half the subjects received irradiated pork and the other half received non-irradiated pork, separated by 5-day washout intervals; the group exposures were reversed during the second 15-day period. There were no adverse clinical effects and no prolongation of prothrombin time for any individual or group following consumption of high-dose irradiated pork.

In a third study, 13 human volunteers consumed a variety of foods irradiated to high doses and stored for three months at room temperature. These foodstuffs were evaluated for acceptability and acute toxicity (429). Rotating menus (three daily menus) supplied approximately 80% of calories from irradiated foods. Potatoes, flour and oranges were irradiated to low doses (0.1–1.5 kGy), while major caloric components of the diet were irradiated to high doses of 25–40 kGy. No clinical abnormalities were noted.

In this series of experiments, designed to detect toxic effects after short latency periods and after a one-year latency period, humans consuming high-dose irradiated diets for 15-day intervals showed no toxic effects either during the feeding interval or at subsequent follow-up evaluations. In many of the irradiated foods, the authors noted decreased thiamine and ascorbic acid content and detected the presence of increased “browning reaction” derivatives, fat-soluble carbonyl compounds and thiobarbituric acid reactants (presumably an index of lipid peroxide formation). Significantly, in the study involving 32% calories derived from high-dose irradiated pork stored for one year at room temperature, neither prothrombin time nor any other clinical laboratory parameters was altered. The authors concluded that in all of these studies the digestibility of macronutrients was similar in control diets and in the high-dose irradiated diets. Ideally, human safety studies require double-blind experimental designs to avoid either placebo bias or unintentional experimenter bias. Volunteer participants in these studies, however, reported in journal entries and interviews that control foods and high-dose irradiated foods could be readily distinguished by flavour, odour and texture. Earlier studies on the acceptability of irradiated foods reported that the volunteers noted differences in the colour of strawberries and powdered milk, in the odour of ground beef and in texture changes in fruits and vegetables (308). In addition, foods from the cereal product group (bread, crackers, macaroni, pound cake and rice) irradiated to high doses were readily distinguished from the non-irradiated items (430). The authors noted that in practice these foods would be irradiated to much lower doses for control of insect infestation, which would result in a minimal difference between the irradiated and non-irradiated items. Significantly, no adverse clinical experiences or reactions associated with consumption of irradiated foods were reported by volunteers. Clinicians detected no adverse findings from physical evaluations or in clinical laboratory values made either during or after these short-term exposures. These studies, however, were not designed to detect long-term nutritional deficiencies or the potential for carcinogenic effects related to the consumption of high-dose irradiated diets.

6.6 Conclusions

Sections 3–5 on chemistry, nutrition, and microbiology addressed many of the early concerns and identified critical elements necessary for good food irradiation practices. This section has presented information from several studies of irradiated foods carried out in the 1950s and 1960s, many of which were processed under conditions that would not be considered as having followed current “good irradiation practice”. Nevertheless, this extensive collection of data demonstrates that irradiated foods using a variety of sources under a variety of conditions are toxicologically safe. The carcinogenicity and mutagenicity studies with irradiated food and feed have not demonstrated any treatment-related effect.

Based on the body of toxicological data reviewed here, the Study Group concluded:

- Food irradiation is toxicologically perhaps the most thoroughly investigated food processing technology.
- Animal studies are suitable models and predictions from them are supported by human studies.
- The sensitivity of the methods used to assess safety is adequate, and many studies purposely used higher doses and larger amounts of irradiated food in an attempt to elicit a positive response.
- The large number of toxicological studies, including carcinogenicity bioassays and multigeneration reproductive toxicology evaluations, did not demonstrate any short-term or long-term toxicity related to the process.
- With the exception of a few easily rationalized positive results, the highly diverse and sensitive mutagenicity studies on a variety of foods, including radiation-sterilized chicken, are overwhelmingly negative.
- Foods that are appropriately prepared, packaged and irradiated to high doses under proper conditions to sterilize them should be deemed safe.

7. Packaging considerations

7.1 Introduction

In view of the important role packaging plays in facilitating irradiation processing, in protecting irradiated food from recontamination and in maintaining the quality of the food, it is essential to consider the influence of irradiation on packaging materials. If the packaging is to be effective, then the irradiation should neither compromise the functional properties of the packaging material nor facilitate the migration of any undesirable components from the material into the food.

7.1.1 **Objectives**

The Study Group's objectives were:

- To reassess the safety of flexible packaging developed in the 1950s and 1960s (431, 432) and currently employed for radiation sterilization of food in the light of current knowledge.
- To reassess quality assurance methods for flexible packaging, also developed in the 1950s and 1960s, and to recommend improvements that may be needed.
- To assess the suitability of all available packaging materials for use in high-dose applications of food irradiation and, accordingly, to recommend the best candidate materials and processes for the development of future generations of packaging for radiation-sterilized food.

This section focuses on flexible packaging, manufactured from polymers, which is technologically and economically suitable for the purpose of packaging precooked foods to be radiation sterilized.

Products and processes can be assessed by two routes, good engineering practice and strict reliability practice, which are complementary rather than contradictory.

Good engineering practice. Unless proven otherwise, every component or operation is admissible when well accepted and documented practices are followed. Further, any previous data on similar products and processes are regarded as suitable and reliable unless proven otherwise.

Strict reliability practice. Every component or operation is considered apt to fail until assessed otherwise to the desired level of confidence. Further, any previous data on similar products and processes must be strictly assessed to determine their relevancy, accuracy and reliability.

Strict reliability practice is customarily followed for high-technology processes and products, where public acceptance or tolerance of failures is doubtful. Owing to the current status of public acceptance, food irradiation is one such process, and irradiated foods and associated packaging are such products.

7.1.2 **The effects of radiation on macromolecules**

In irradiating prepackaged food, which comprises different types of macromolecules, the goal is to maximize damage to the DNA of contaminating bacteria and to minimize damage to structural polymers of the packaging.

This seemingly self-contradictory goal can be accomplished if at least one of two conditions is met:

- the radiation durability of the two types of macromolecules is substantially different;
- the definition of damage threshold for the two types of macromolecules is substantially different.

The interactions of radiation with materials and the consequent chemical changes are comprehensively discussed elsewhere (443, 444); (see also section 3). The information given here addresses the primary processes only briefly and focuses on the final chemical effects, their manifestations and practical implications.

The interactions of ionizing radiation with matter take place via transfer of energy to the electrons in atomic or molecular orbitals, resulting in their displacement. This displacement can eventually result in bond scission, which is the main concern with respect to the damage to polymers. Since most commonly used polymers comprise primarily carbon, hydrogen, nitrogen and oxygen atoms and have molecular orbitals of similar size, their durability to radiation can be classified in a simplified way according to the nature of these orbitals. Those molecular orbitals associated with the polymer backbone play the major role in the resistance of polymers to scission. The radiation durability of these orbitals serves as a general ranking of the radiation durability of polymer families, which in decreasing order is:

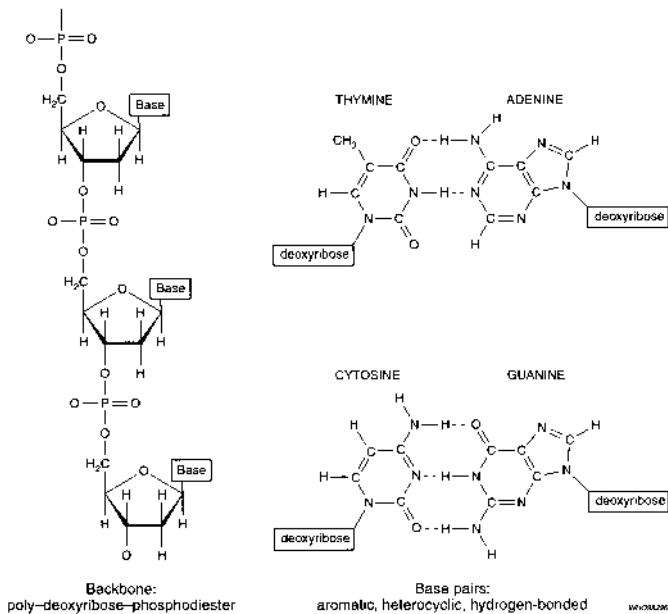
- polymers containing aromatic groups in the backbone, e.g. polyethyleneterephthalate (PET), polyimide (PI), poly[aryl-ether-ketone] (PEEK), etc.
- polymers having an aliphatic-chain backbone with aromatic side groups, e.g. polystyrene (PS), etc.
- polymers having an aliphatic-chain backbone containing ester or amide groups, e.g. polyamides, polyesters, polyurethanes, etc.
- polymers having a simple aliphatic-chain backbone, e.g. polyethylene (PE)
- polymers having an aliphatic-chain backbone with side groups containing various atoms, e.g. polyvinylchloride (PVC), polyvinylalcohol (PVA), polyvinylfluoride (PVF), but with the exception of polytetrafluoroethylene (PTFE), which is relatively sensitive to damage
- polymers having an aliphatic-chain backbone with double-bond side groups, e.g. polymethylmethacrylate (PMMA)

The damage caused by irradiation also depends on the structural robustness of the polymer. Ring structures, ladder structures, crystalline moieties and inter-chain interactions all decrease the mobility of chain segments, thus increasing the probability for recombination of scissioned chains. For packaging to be used in the radiation sterilization

of prepackaged food, the preferred polymers are those comprising groups associated with high radiation durability and characterized by strong inter-chain interactions or high crystallinity.

The primary target in the radiation sterilization of food is the DNA of foodborne bacteria. When this is damaged, the bacterium is eliminated within a few cell divisions. The molecular weight of DNA far exceeds that of all other molecules in the living cell; hence, its energy absorption is the highest. Although this unique molecule (435) is highly durable to gross radiation damage, owing to its aromatic groups, heterocyclic rings, hetero-atom rich backbone, and the double-helix structure bridged by a multitude of hydrogen bonds (Figure 18), certain base moieties can be affected, possibly leading to rupture of a sugar-phosphate linkage in a single strand. The radiation durability of DNA, particularly in the low-moisture environment within a spore, means that high doses of radiation are required to achieve sterilization, even after the heat pretreatment given to inactivate proteolytic enzymes. However, it is possible to attain the goal of damaging bacterial DNA without adversely affecting the food or the packaging, since there is a difference in the concept of damage threshold for the two types of macromolecules, (DNA and packaging polymers).

Figure 18
Components of the DNA molecule^a



^a Reproduced from Stryer (435) with the permission of the publisher.

Minimum damage threshold to the DNA polymer can be taken as “the number of double strand breaks sufficient to reduce the total count of living bacteria from N to $N \times 10^{-12}$ counts/g”. However, in practice, only the destruction of spores of proteolytic strains of *Clostridium botulinum* in low-acid shelf-stable food is considered. Maximum damage to the packaging polymer can be taken as “the number of scissions required to change the mechanical properties by 10% and/or to reach the allowed total amount of extractives”.¹

Numerous factors that affect the radiation damage threshold of DNA, such as moisture, dose, dose rate, atmosphere, temperature and pH, also affect the radiation damage threshold of the packaging polymers. The term “damage” is used to describe both degradation of the polymer matrix and the formation of extractives, since both phenomena stem from scission of the polymeric chains and side-groups. The important factors affecting damage to packaging are:

- Total dose. The damage–dose correlation depends on the total dose and, hence, for a reliable selection of packaging polymers it is necessary to determine their actual damage–dose profile (25, 436, 437) in the relevant dose range. In polymers that are highly durable to radiation, only negligible changes in properties are measured. However, the accuracy and validity of these measurements are questionable. In accordance with strict reliability practice, a non-finding cannot be regarded as a positive proof unless a reasonable safety margin has been incorporated in testing to compensate for the great uncertainty in the results. The formation of extractives in irradiated polymers is more discernible and may be a better guide in assessing their radiation durability.
- Dose rate affects chemical processes taking place according to second order (and higher) kinetics, such as radical recombination. For the sake of worst-case analysis, packaging polymers should be tested to their damage threshold at a dose rate that is low enough to ensure that the damage is independent of dose rate.
- Atmosphere. Oxygen reacts readily with radicals and other radiation-produced reactive species, thus promoting radiation-generated damage. This means that oxygen must be removed from the food and headspace prior to sealing the packaging: this is usually done with a vacuum pump. Vacuum removal of oxygen assisted by flushing (e.g. with carbon dioxide) may be practised. Food packaging polymers should exhibit both low oxygen content and low permeability to oxygen.

¹ Extractives are molecules capable of diffusing within the polymer that when near or on the surface of the polymer can be transferred into a contacting substance, e.g. solvent or food.

- Thermal and mechanical history. Fabrication of polymer sheets and laminates involves extensive thermal and mechanical processing that can give rise to chemical degradation and latent stresses, affecting the radiation durability of the polymers (436). Recycling of materials for use in packaging should therefore receive special attention.
- Irradiation history. Pre-sterilization of food packaging by irradiation should be avoided or at least properly documented and its consequences assessed.

A check list is one of the primary tools in the reliability assessment of polymeric packaging for irradiated food. It should contain each and every factor that could cause concern at the theoretical level. Each factor should be examined singly, and the possibility of synergistic effects from several factors acting together should be investigated. An example of such data compilation is shown in Table 33.

Table 33

Model quality assurance check-list for a candidate polymer

Group	Factor	Data		Durability assessment	
		Full/part/NA	Reliability	Theoretical	Practical
Polymer	Family				
	Producer				
	Specific brand				
	Additives				
	MW distribution				
	Linearity				
	Crystallinity				
History	Processing				
	Thermal				
	Mechanical				
	Irradiation				
Irradiation	Dose				
	Dose rate				
	Atmosphere				
Food	Type				
	Absorption (g/g)				
	Swelling (cm/cm)				
Durability	Tear (before/after)				
	Puncture (before/after)				
	Abrasion (before/after)				

MW = molecular weight; NA = not applicable.

7.1.3 *Packaging characteristics*

- **Extractives.** Molecules of low molecular weight and high diffusivity that can diffuse within the packaging polymer and can be extracted from it into the food. These extractives may be residual compounds from the polymerization process, additives to the polymer or degradation products from the mechanical and thermal processing. For food packaging materials that have already been approved, only molecules either formed or released as a result of irradiation are relevant to the evaluation. The quantity of extractives can be determined by well-accepted protocols, before and after irradiation. Their toxicity is more difficult to assess.
- **Packaging integrity.** Particular attention should be paid to the packaging walls (e.g. for puncturing), sealing areas, and intra-laminate adhesion. Double packaging may provide extended protection of the layer in contact with the food and may also eliminate the need to use laminates. For sealing, welding appears to be much safer than glueing. The durability of seals needs to be tested with respect to the combined effect of mechanical loads, heat and radiation. Well-established food packaging materials, with documented testing and market experience (including sealing and lamination), are preferred. Some of these have already been radiation-tested for other purposes and thus only the combined effects need to be tested.
- **Packaging permeability and swelling tendency.** Extremely low permeability and swelling tendency of the food packaging polymers are required for their long-term reliability as oxygen and water barriers. These characteristics should be tested before and after irradiation as part of the polymer screening process.
- **Packaging additives.** A wide variety of proprietary additives are commonly present in polymer films and their use is not always documented. Of particular interest are aromatic antioxidants that are potentially toxic.
- **The food-contacting layer.** In the multilayered structures that are likely to be needed to satisfy the demands of radiation processing of prepackaged food (438), the layer in contact with the food should be the one most strictly tested as to the formation and migration of potentially toxic compounds.

7.2 **The database**

7.2.1 *Radiation durability of polymers*

A literature survey was carried out with particular emphasis on high-dose (above 10 kGy) applications relevant to the radiation sterilization of food. Unfortunately, most of the literature relates to lower doses and

to packaging polymers of relatively low radiation durability. A database on radiation durability of polymers has been compiled at the Soreq Nuclear Research Centre, Israel, for use in assessing the durability and functional reliability of polymeric materials in space applications (439, 440) and in the packaging of irradiated food (441). This database focuses on the reliable selection of highly durable polymers for use in the envelope regions of low-earth-orbit satellites, where the total radiation dose may exceed 50–100 kGy. One of the basic documents in this compilation is the Harwell database on the durability of polymers to ionizing radiation (442). The data it provides (Table 34) are regarded only as indicative, since variation in the initial polymer composition may give wide variation in the properties of the same nominal polymer.

For space systems, which are exposed to intense radiation, polymers highly durable to radiation are commonly used (443–449). Not surprisingly, most of these polymers are commercially available and some of them are extensively used for food packaging in light of their robustness and long-term reliability. Some of these polymers are listed in Table 35.

An important database on the radiation durability of polymers has been assembled over three decades by researchers at the Institut für Strahlenhygiene des Bundesgesundheitsamtes [Institute for Radiation Hygiene, Federal Office for Health (BGA)], Germany (450–453). A compilation of the ionizing radiation effects on some food packaging materials is presented in Table 36 (452).

Other data relevant to the irradiation of food packaging materials are also available (454–457).

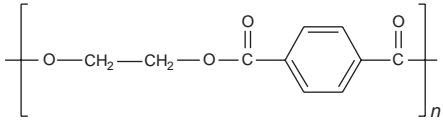
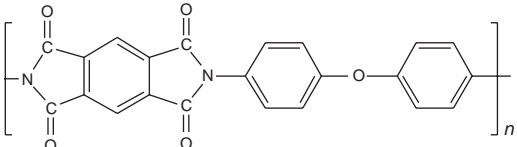
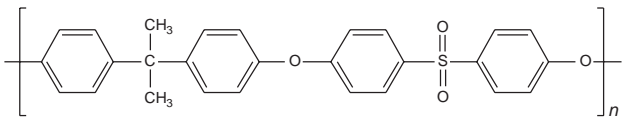
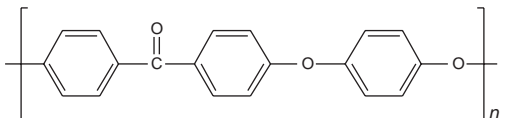
Finally, the data compiled by the United States Army Natick Research, Development and Engineering Center cover all the experimental work and theoretical assessment carried out by or for the United States Army for the purpose of providing reliable and safe radiation-sterilized prepackaged food. Selected documents containing these data are

Table 34
Selected data on the radiation durability of polymers

Polymer	Radiation threshold (kGy)	
	Some damage	Severe damage
Polyethylene	100	2000
Polytetrafluoroethylene	5	40
Fluorinated ethylenepropylene	50	500
Polyvinylidenedifluoride	100	1000
Polystyrene	700	>10 ⁴

Table 35

Selected data on the radiation durability of polymers for space applications

Polymer	Radiation threshold (kGy)	
	Some damage	Severe damage
Polyethyleneterephthalate 	$1-3 \times 10^3$	1×10^5
Polyimide (aromatic) 	$>2 \times 10^3$	$4 \times 10^4-3 \times 10^5$
Polysulfone 	6×10^3	$>10^4$
Poly[aryl-ether-ketone] 	$>1 \times 10^5$	$>10^5$
Epoxy resins (aromatic)	3×10^5	–
Polyurethane (aromatic)	–	5×10^4
Silicone resins	3×10^5	–

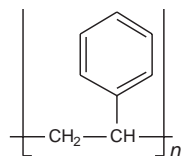
Source of data: Bouquet (443), DuPont (444), Bouquet et al. (445), Meyer et al. (446), Bouquet et al. (447), Coulter et al. (448), Funk & Sykes (449).

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Table 36

Selected data on the radiation durability of food packaging

Polymer	Radiation effects
Low-density polyethylene; medium-density polyethylene; high-density polyethylene	<p>Antioxidants are mandatory for conservation of mechanical properties upon irradiation (10–25 kGy)</p> <p>Antioxidants are extracted from these polymers</p> <p>The amount of volatile products formed depends on the formulation and processing history of the sample</p> <p>More than 100 volatile compounds have been identified, including small amounts of benzene and its derivatives</p> <p>Radiation dose for 50% decrease in elongation at break (for high-density polyethylene): 6 kGy without stabilizer, up to 36 kGy with stabilizer</p>
Polypropylene	<p>75% decrease in elongation at break at 10 kGy (irradiated in oxygen)</p> <p>Antioxidants are mandatory for conservation of mechanical properties upon irradiation</p>
Polyamide-6	50% increase in acetic-acid extractives at 60 kGy (irradiated in oxygen). Extractives include monomer and oligomers
Polyethyleneterephthalate	<p>Negligible, insignificant or unmeasurable changes in all parameters, including all extractives and permeability, at dose ≥ 56 kGy</p> <p>Extractives are 30 times lower than with polyamide-6</p>
Polystyrene	<p>Negligible, insignificant or unmeasurable changes in all parameters, including polar extractives, at dose ≥ 56 kGy</p> <p>Non-polar extractives (<i>n</i>-heptane) increase by 7–18 times.</p> <p>Extractives include monomer and oligomers</p> <p>No substantial differences in tensile, burst and seal strength are observed at dose ≥ 60 kGy</p> <p>No significant changes in quantity and composition of extractives are observed at dose ≥ 60 kGy</p> <p>Polyamide showed marked reduction in tear resistance</p>
Antioxidants (phenol and organo-tin compounds)	<p>Commercially added to polymers with aliphatic backbone^b to increase ageing resistance</p> <p>All additives migrate, and are mostly toxic</p> <p>Extractives may be affected by radiation, depending on polymer, radiation mode and extraction liquid</p>



Source of data: Bögl et al. (450-453) with the permission of the publisher.

^a Comprising high-density polyethylene, polyamide-6, polyethyleneterephthalate.

^b Polyethylene, polypropylene, polyvinylchloride, polystyrene.

available (437, 458–460). Of special interest is the reasoning used in the development and fabrication of the flexible pouches as described by Pyne et al. (458). The food contactant material was primarily selected on the basis of maximal lamination durability of available polymers using available lamination techniques, rather than on maximal radiation durability.

7.2.2 **Extractives**

Only a few articles in the literature address in any substantial way the radiolytic formation of extractives (461–463). The data pertain primarily to polymers used in the 1950s–1970s, and to a lesser extent to new polymers that are candidates for the next generation of food packaging (Table 37). The two primary sources of extractives are: trunk polymer fractions and their radiation-generated fragments, which are documented in the literature; and additives and their radiation-generated fragments, which are seldom reported or specified and which remain to be tracked in the course of the quality assurance process.

The literature on materials for space applications provides a useful source of data on extractives in radiation-stable polymers. The primary goals for these materials are high durability, high reliability, insignificant changes of performance upon irradiation, and extreme cleanliness as expressed in terms of outgassing levels (American Society for Testing Materials, standard ASTM E-595). Not surprisingly, many high-quality polymer films manufactured nowadays meet these strict specifications, including films made from polyvinylfluoride (PVF), polyvinylidene-difluoride (PVDF), polyethyleneterephthalate (PET), aromatic polyimide and others. It is noteworthy that the utmost cleanliness of these polymer films does not stem from customers' needs but rather from manufacturing requirements aiming for flawless extrusion into films.

The selection of polymers suitable for the packaging of radiation-sterilized precooked food depends ultimately on minimizing extractives, natural and radiolytically-formed alike. Furthermore, proof derived from analytical and/or animal feeding studies that such extractives are non-toxic is essential and is valid only for the polymers *per se*. If polymers containing additives are considered, their selection should be made only after judiciously taking into account the extractives resulting from the irradiation of the entire system of polymer/additives/food. However, additive-free polymers are preferred.

From the analytical standpoint, the sensitivity of detection has dramatically increased over the past four decades, so that particular attention should be given to newly reported levels of extractives from food packaging. Fortunately, these data are being routinely accumulated by

Table 37

Selected data on extractives in food packaging candidate polymers^a

Polymer	Extractives	
	Pristine	Radiation induced
Low-, medium- and high-density polyethylene	Antioxidants and other additives	Degraded antioxidants and other additives Polyethylene oligomers (small quantities) and their oxygenated derivatives 100 volatile compounds, including alkanes, alcohols, aldehydes, ketones and acids
Polyamide	Unreacted monomer	Slight increase in extractives as compared to the control
Polyethyleneterephthalate	No detectable extractives	No detectable extractives Some inorganic gases, e.g. carbon dioxide
Polystyrene	Polystyrene oligomers (small quantities)	Polystyrene oligomers (small quantities)
Polyimide	* TML < 1% CVCM < 0.1%	* TML < 1% CVCM < 0.1%
Polysulfone	* TML < 1% CVCM < 0.1%	* TML < 1% CVCM < 0.1%
Silicon resins (RTV, spacegrade)	* TML < 1% CVCM < 0.1%	* TML < 1% CVCM < 0.1%

CVCM, condensable volatile cumulative mass on a counter-plate at 25 °C (American Society of Testing for Materials); TML, total mass loss at 125 °C 10⁻⁶ torr; RTV, room temperature vulcanized rubber.

Source of data: Rojas de Gante & Pascat (461), Tripp (462) and Killoran (463).

^a Items marked with an asterisk are space-qualification data.

the leading manufacturers of food packaging, as part of their standard quality assurance practices. Extrapolation of their extractives-testing routines to irradiated packaging products seems customary. Further extrapolation may be needed for testing the final packaging products, in particular laminates and pouches, rather than raw films.

From the regulatory standpoint, levels of extractives in food that were tolerated in the 1960s and 1970s may no longer be considered safe. However, the natural levels of potentially toxic materials in commonplace foods can be considered safe, and standards for food packaging safety must be adjusted accordingly. The growing development of new generations of food packaging materials takes these new trends in safety assessment into account.

Animal feeding experiments (460) were used to confirm the safety of packaging for radiation-sterilized foods. An extensive and comprehen-

sive study on irradiated chicken (9) demonstrated that the consumption of the high-dose, radiation-sterilized food, as a reasonable proportion of the diet, does not pose any health risks. The many subsequent years of hazard-free consumption by human customers have given further support to the safety of this line of products.

An interesting issue arises relating to the trays made of polystyrene (PS) foam (styrofoam) commonly used for prepackaged foods. PS has an aliphatic backbone that is stabilized by the aromatic side groups. It is much more durable to radiation than the widely used PE, but somewhat less durable than PET. It has been tested for radiation-sterilized food packaging at doses up to 56 kGy, but with no safety margin, and small amounts of extractives have been detected (however, doses up to 600 kGy have been applied in tests on its reliability for use in calorimeters for dosimetry). Accordingly, PS is most probably safe for use up to 56 kGy, but a damage-dose profile for PS in the dose range above 60 kGy should be determined.

From a mechanical standpoint, PS trays function faultlessly following food irradiation. One way to circumvent the issue of potential extractives is to laminate styrofoam trays with PET. However, the already available lightweight PET trays would be a more affordable solution for applications involving radiation sterilization of foods in trays.

7.2.3 ***Radiation-effected permeability***

There is a considerable body of literature relating to radiation-induced modification of polymer permeability, either through graft-copolymerization or neutron beam-produced tracks. It is recognized that radiation can affect the oxygen permeability of food packaging polymers (452). In the case of low-density PE, the oxygen permeability may significantly increase upon irradiation to 25 kGy, in contrast to high-density HDPE or PET in which there is no appreciable increase. However, in practice, the packaging for radiation-sterilized food typically contains a barrier middle layer made of aluminium film. Consequently, the gas permeabilities of the bare polymer films are of minor importance.

Of greater importance is the permeability of the food-contacting layer to migrants that might possibly be extracted into the food. Possible radiation-effected permeabilization of this layer, typically ignored in the literature, needs to be considered. A significant radiation-generated mechanical degradation will most probably precede changes in free volume and in associated permeability. Hence, polymers whose permeability is increased by radiation will be rejected on grounds of mechanical failure. If the selected packaging polymer is free of extractives, either pristine or radiation-generated, the permeabilization

problem is no longer a cause for concern. However, if a compromise is made and the selected polymer is a possible source of extractives, the radiation-effected permeabilization factor may need to be addressed experimentally.

7.2.4 **Food interactions with packaging**

Data are available on food interactions with packaging in the general food packaging literature, but there are few specific studies related to radiation-sterilized foods. Taint-transfer – the transfer of odours from packaging into the food – is an important issue (464). The olfactory sense is extremely sensitive in humans and may detect traces of volatile migrants that are at levels lower than all relevant safety thresholds and that might be undetectable by most instruments. These odours compromise the quality of the food, which could present a serious commercial problem and undermine acceptance of irradiated foods by the general public. The taint-transfer of selected polymers is summarized in Table 38. Since the radiation doses in all cases were lower than 4 kGy, the results are only indicative.

7.2.5 **Specific packaging for irradiated food**

The vast majority of the available data relate to packaging materials and methodologies for radiation-sterilized prepackaged foods that were developed by or for Natick in the last four decades. A reasonable proportion has been published or appears in petitions to the FDA. Unpublished material in this comprehensive database, some of which is discussed elsewhere in this section, can be accessed on request.

7.3 **Industrial packaging for irradiated food**

7.3.1 **Polymers commonly employed**

Most of the polymers covered in the database are commonly employed for various types of food packaging. There has been, however, a distinct shift away from the packaging materials used 20–40 years ago, when

Table 38
Data on the taint-transfer of selected polymers (dose < 4 kGy)

Polymer	Taint-transfer observation
Low-, medium- and high-density polyethylene	No evidence for taint-transfer was found
Polyamide	None
Polyethyleneterephthalate	Some indication of taint-transfer
Polystyrene	Evidence for taint-transfer

most of the relevant research activities took place and the data were compiled. There are two primary reasons for this shift: the availability of a large variety of new polymers and polymer-grades with improved performance of strength, barrier properties and ageing durability; and the increasingly strict demands imposed by customers, and consequently by manufacturers, regarding quality and preservation of packaged food. These demands have fuelled the on-going search for improved packaging materials.

Most materials used for food packaging in the 1950s and 1960s were paper products, cellulose derivatives (cellophane), rubber derivatives, polyethylene, polypropylene and polyvinylchloride. Various types of waxes, rubber products and vinylidene chloride copolymers were used as coating materials to enhance barrier properties. Polyamide films have been used as a barrier constituent of laminates to reduce their permeability. Aluminium foils have also been introduced into food packaging to impart impermeability.

In the course of time, the well-known polymer PET began to be produced in a wide variety of grades that enable facile extrusion, blow-extrusion and heat-sealing. This polymer exhibits excellent mechanical, barrier and durability properties. Furthermore, it has been successfully manufactured to a very high purity and has been found to be practically free of extractives. Demand for PET and its laminates has therefore grown extensively over the last 20 years. The subsequent increase in production capacity has resulted in scaling up the processes and, consequently, a dramatic reduction in product costs and prices. This has further increased its use in food packaging, especially for bottled carbonated drinks; the robustness of PET in withstanding high pressure, rough handling and extraction in aggressive solvents is exemplified by the huge numbers of bottles of such drinks sold daily.

Another newly introduced highly durable, well-known polymer is the aromatic polyimide, which is also produced in a wide variety of grades. Although it is rarely employed for food packaging in view of its relatively high price, it is widely used in the electronics industry because of its electrical properties and its durability to a wide variety of hazards. A variety of polyimide grades has been made available to this industry to satisfy diverse needs: rigid and flexible printed circuit boards, single- and multi-layer printed circuit boards, etc. The space industry also makes extensive use of polyimide in many applications, including as external thermal blankets. In this application, the polyimide films are expected to function reliably, despite high mechanical loads and extensive exposure to photons in the far ultra-violet range and to high doses of ionizing

radiation. The space industry has established the radiation durability of both aromatic polyimide and PET to doses higher than 1000 kGy.

Laminated polymeric packaging materials are widely employed for many foods, e.g. snacks, fish-products, juices, etc., in order to satisfy the combined needs of high mechanical durability and impermeability. Various lamination technologies are in common use, with or without the use of adhesives. Corona pretreatment of the surface (an electric discharge technique that generates plasma, resulting in slight surface oxidation and chain scission) is commonly used to improve printing as well as lamination quality.

Polymer films currently manufactured for high-technology industries meet strict specifications of purity, durability and reliability. The utmost cleanliness of the resin necessary for the extrusion of high-quality films assures migrant-free polymers. Such polymers are crucial not only for space applications, but also for advanced electronics, micro-optics and integrated electro-optical systems. In these systems, any extractives, either “native” or generated by degradation (e.g. laser-generated degradation), are detrimental and could compromise the operation of most devices.

7.3.2 ***Polymers highly durable to radiation***

Polymers with high durability to radiation are commonly used in the nuclear and irradiation industries, as well as in space industry systems, including satellites and space vehicles. All these environments are characterized by very high fluxes of ionizing radiation. The total doses accumulated by materials in these environments may exceed MGy and GGy levels in relatively short periods of service time.

In all these environments, polymeric materials are used satisfactorily. Their use in space is steadily increasing, replacing the use of metals and ceramics, because of the severe weight restrictions. The need to reduce weight drives the development of new lightweight, polymer-rich systems and has necessitated the identification of existing polymers that are highly durable to radiation.

Following almost four decades of space research, sufficient knowledge and experience have been gained to direct engineers in their selection of polymers for space applications. The most commonly used highly radiation-durable polymers in space applications are listed in Table 35. Among these, the most adequate for food packaging are the first two, PET and aromatic polyimide, which are widely used and commercially available in the form of films.

7.3.3 **High-barrier packaging polymers and laminates**

The chemical features of high-barrier polymers can in general be characterized as highly aromatic, highly polar, highly linear, and of high molecular weight. The combination of polar and non-polar (hydrophobic) interactions imparts to the polymers not only radiation tolerance but also strong inter-chain interactions that are associated with low permeability.

Many foods, like cooked meat, comprise both aqueous (hydrophilic) and fatty (hydrophobic) ingredients, as well as some oleophilic (emulsifying) ingredients. This “cocktail” acts as a swelling agent for polymers; therefore polymers used in packaging must be resistant to it over the desired storage time and temperature range. For military use, and uses of similar complexity, it is difficult to impose limitations on storage duration and temperature. Therefore, packaging barrier material that is most resistant to swelling should be used. Aluminium foil is commonly used for this purpose, typically as a middle layer in a three-layer laminate. Once a puncture-free aluminium foil is laminated into the packaging, the long-term barrier requirements are met. Such laminate technology has been validated over several decades, and is widely utilized for common food products. It has already been successfully used for the packaging of radiation-sterilized prepackaged food, and its use is likely to continue.

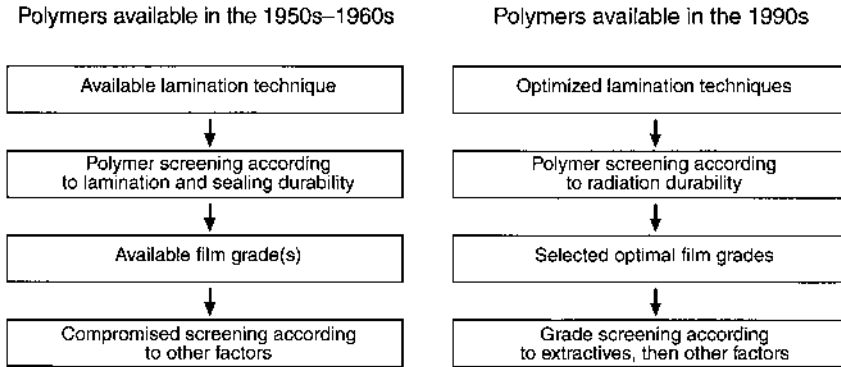
Newly developed alternatives to the traditional barrier packaging that offer even more advantages are currently under evaluation for performance, durability under service conditions, and long-term functional reliability (465). These include:

- metal-free barrier laminates, having extremely low oxygen and water permeation, made with a glass-like barrier layer produced by plasma-enhanced chemical vapour deposition in the laminate;
- packaging materials containing an oxygen scavenger in the polymeric films, which can react with residual oxygen in the packaging and thus eliminate oxidation-induced food spoilage;
- packaging materials containing antibiotics in the polymeric films, which can prevent or retard growth of residual bacteria and fungi in the food.

The selection of polymers for laminated packaging currently used for radiation-sterilized food was based primarily on long-term stability of the lamination and sealing (458, 460), polyethylene being the only choice. While this selection was justified at the time new concerns about dose-safety margin and testing of extractives emerged, leading to further research efforts. Nowadays, numerous flexible and heat-sealable grades of radiation-durable polymers are commercially available to the food,

Figure 19

Comparison of polymer selection in the 1950s–1960s and the 1990s



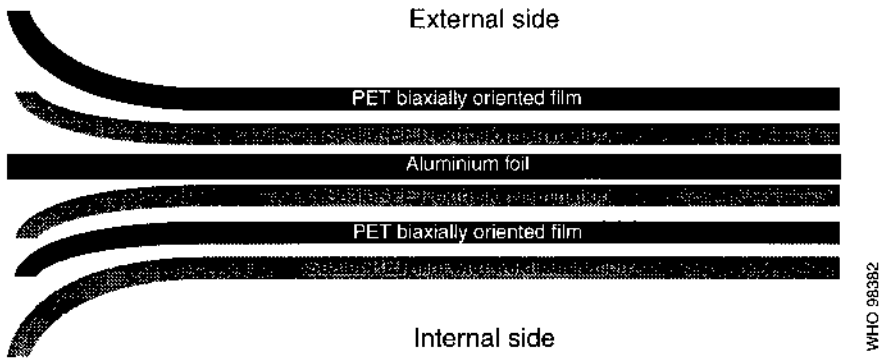
electronic and space industries, so that polymer properties can be selected according to particular needs. This tailoring and optimization can be achieved without compromising either the polymer constitution or consequent radiation durability. Conditions are now such that it should be possible to produce an unquestionably safe and highly durable packaging laminate for high-dose radiation-sterilized prepackaged foods (Figure 19).

7.3.4 Current practice in high-reliability food packaging

- The commonly used heat-sealable PET is an amorphous PET copolymer (melting point as low as 80 °C); the PET grade used for bottling soft drinks is partly crystalline, and those used for high-strength films and fabrics are highly crystalline.
- All grades of PET films have been given full approval for use with all types of food by regulatory agencies in many countries.
- Aluminium foils with certified low oil content are available for laminates.
- PET laminations using biaxially oriented films require the use of adhesives. Epoxy adhesives, which are radiation-durable, are rarely used for regular food-packaging laminates owing to their price.
- Heat-sealable PET is adequate for either welding or extrusion lamination, in which molten polymer is applied in the form of a thin film between the layers to be laminated.
- The quality of the raw films is tested by their manufacturers who are required to provide a certificate of compliance certifying their adequacy as a food-grade material.
- Based on these facts, a suggested organization of the laminate for prepackaging foods to be irradiated is shown in Figure 20.

Figure 20

Organization of a laminate for prepackaging food to be irradiated



PET - polyethyleneterephthalate

7.3.5 Packaging for radiation-sterilized precooked food

The current practice in packaging for radiation-sterilized precooked foods relates to materials and methodologies that were developed and tested by or for Natick over the last four decades. These foods have been produced for consumption by special groups in the United States and South Africa. The accumulated experience of use, especially by NASA astronauts and their Russian counterparts in joint space flights, is considerable (see Annex 1).

New developments in packaging for radiation-sterilized foods are in progress with the aim of diversifying military ration components and further improving their sensory attributes and overall reliability. These developments include the assessment of improved barrier layers, improved food preparation and packaging technologies, and enhanced quality assurance methodologies. Future packaging trends under assessment include a single-layer food pouch of highly radiation-durable polymers, either in a barrier laminate or used alone. The assessment will be based in part on the physicochemical responses and in part on the analysis of extractives, rather than on mechanical changes. It will include determining damage-dose profiles for many representative polymers as candidates for radiation-processed food packaging.

A commercial company in South Africa (BIOGAM) uses a packaging technology similar to that developed by Natick. Radiation-sterilized foods have been produced for several years and are intended for consumption by South African military personnel as well as by hikers, backpackers and yachtsmen (see Annex 1). The data on the currently

used packaging materials for radiation-sterilized foods in South Africa is proprietary, but can be accessed on request.¹

7.3.6 **Polymer packaging quality assurance**

Plastics used to be regarded as a cheap alternative to, or imitation of, an expensive high-quality material such as metal, wood or glass; they represented a compromise on quality in order to gain a price reduction. Only in recent decades have the concepts of quality management and quality production pervaded all industries, including the plastics industry. Nowadays, high-quality resins with accurately specified formulation, properties and history are available from leading companies and dealers. Similarly, high-quality films, laminates and pouches made from them are available from leading producers and used by leading food manufacturers.

However, materials of inferior quality might still be encountered that could compromise the quality of radiation-sterilized prepackaged food. The selection of the appropriate polymer must be therefore accompanied by a complete quality assurance procedure that includes the following steps:

- Identification of the polymer.
- Determination of its desired nominal properties and the limits of allowed deviation.
- Quality inspection of the resin manufacturing process and the product testing process.
- Quality inspection of both manufacturing and testing processes for polymer films and laminates, and for the trays, lids and pouches made from these.
- Quality inspection of the food packaging process and the product testing process.
- Quality inspection of the radiation-sterilization process and the product testing process.

All these steps may be excessive for good engineering practice, but are necessary for strict reliability practice.

7.4 **Regulatory aspects**

Regulations relating to food irradiation and packaging for irradiated foods encompass the following:

- National regulations that permit radiation treatment of specific foods or food products for public consumption.

¹ Ms Ingrid de Bruyn, Atomic Energy Corporation of South Africa, P.O. Box 582, Pretoria 001, South Africa.

- National regulations that permit the use of specific packaging materials for radiation-treated prepackaged food for public consumption.
- Good manufacturing practice methodologies for food production, irradiation, storage and testing.
- Labelling in conformity with the regulations, including the irradiation logo and an appropriate statement.

These legislative aspects are just part of the quality assurance system necessitated by a high-technology product.

A comprehensive, updated database including information on regulatory status is maintained by the Secretariat of the International Consultative Group on Food Irradiation. A listing of national approvals for packaging materials is given in Table 39 (466).

The assessment of the currently used packaging for radiation-sterilized food as safe has been validated by extensive animal feeding studies and several decades of human consumption. However, the safety testing of new discrete packaging materials for irradiated foods could be refined. A comprehensive analysis of volatiles and other extractables would be appropriate and practical (461–463). Laboratories are now equipped with highly improved analytical methods as compared to the 1950s and 1960s, and extensive databases on the toxicity of molecules are available.

7.5 Safety, reliability and suitability assessment

The process of assessing and validating candidate polymeric packaging materials for radiation-sterilized food comprises two steps:

- Theoretical assessment of the suitability of candidate polymers or laminates for the specific application. The durability, safety and reliability of each candidate material are assessed, and safety margins for the applicable radiation dose are set. Applicable definitions of damage thresholds are also established. Data from manufacturer's data sheet for the candidate materials are compared with the stated requirements. Candidate materials considered suitable for the intended application are then recommended for experimental validation and acceptance tests.
- Experimental validation and acceptance tests are carried out on representative lots of specific materials following common statistical sampling and data analysis techniques:

Data-based assessment. This method is comprehensive, accurate and reliable for data related to materials that have already been field-tested. These data may also contain some history of product use, history of storage, and a list of products and packaging solutions. Since

Table 39

Packaging materials authorized for use for radiation-treated prepackaged food^a

No.	Packaging material	Max. dose (kGy)	Country ^b	Date ^b
1	Cardboard	10; 35	UK; Poland	1991 ^c
2	Polyethylene coextruded polyvinylacetate	30	USA; Canada	1988
3	Polyethylene-co-vinylacetate	30	USA	1989
4	Fibreboard	10	India	1997
5	Fibreboard, wax coated (boxes)	10	USA; Canada	1989
6	Glassine paper	10	USA	1975
7	Glass	10	India	1997
8	Hessian sacks	10	UK	1991 ^c
9	Kraft paper	0.5	USA	1975
10	Nitrocellulose-coated cellophane	10	USA; India	1975
11	Nylon 11	10	USA; India	1975
12	Nylon 6	60; 10	USA; India	1975
13	Paper	10; 35	UK; Poland	1991 ^c
14	Paper coated or laminated with wax or polyethylene	10; 35	India; Poland	1990
15	Paper laminated with aluminium foil	35	Poland	1990
16	Polyamide film or polyamide coextruded with polyethylene	35	Poland	1990
17	Polyester-metallized-polyethylene laminate	35	Poland	1990
18	Polyester-polyethylene laminate	35	Poland	1990
19	Polyethylene film (various densities)	60; 35; 10	USA; Poland; India	1975
20	Polyethylene-paper-aluminium laminate	35	Poland	1990
21	Polyethylene-terephthalate	60	USA	1975
22	Polyolefin (low-density as middle or sealant layer)		Canada	1989
23	Polyolefin (high-density as external layer)		Canada	1989
24	Polyolefin film	10	USA	1975
25	Polypropylene sacks	10; 35	UK; Poland	1990 ^c
26	Polypropylene – metallized	35	Poland	1990
27	Polystyrene film	10	USA; India	1975
28	Polystyrene foam trays (Styron 685 D)	10	Canada; India	1989
29	Rubber hydrochloride film	10	USA; India	1975
30	Steel, tin plated or enamel lined	10	India	1997
31	Vegetable parchment	60; 10	USA; India	1975
32	Vinylchloride-co-vinylacetate film	60; 10	USA; India	1975
33	Vinylidenechloride-coated cellophane	10	USA	1975
34	Vinylchloride-co-vinylidenechloride film	10	USA; India	1975
35	Wood	35; 10	Poland; India	1990
36	Viscosa	35	Poland	1990

^a Adapted from reference 466 with permission. Updated by the Secretariat of the International Consultative Group on Food Irradiation, September 1997.

^b Approvals: USA – 1975; Canada – 1989; Poland – 35 kGy, 1986; United Kingdom – 1991; India – 10 kGy, 1996; earliest date of approval is cited.

^c For dry herbs.

currently produced raw materials, although compatible with those used in the past, may differ from the original, new acceptance tests would be mandatory if production is reinstated. While this conservative methodology is best for addressing conservative tasks, it is of limited value when the task at hand involves extrapolating to higher

radiation doses, longer storage durations, and stricter damage and safety thresholds.

Extrapolation-based assessment. Radiation sterilization of prepackaged, precooked food is currently restricted to specific consumers, i.e. patients in hospitals that need sterile food, personnel on military or space missions, and individuals engaged in certain outdoor activities. Approval for general public consumption necessitates establishing that the safety of prepackaged foods radiation-sterilized at doses exceeding 10 kGy is not compromised by the packaging. This process again involves a theoretical assessment and an experimental validation.

The theoretical assessment can be an extrapolation (or interpolation) from data that already support the safety assessment. This is indeed the case regarding the radiation stable packaging polymers, as shown throughout this section.

The experimental validation could be relatively straightforward for newer materials. Fully approved and comprehensively tested food-grade, heat-sealable, commercially available polymers like PET (and perhaps polyimide) could be easily adopted. Extrapolation of their approval is straightforward, and requires irradiation followed by post-irradiation testing for certain mechanical properties and extractives.

Since safety has been demonstrated for foods packed in a trilaminate pouch with a low-density PE food-contacting layer, irradiated to doses as high as 105 kGy, products irradiated between 10 kGy and doses consistent with microbial safety and sensory acceptance would be correspondingly safe. If the damage–dose relation of this food-contactant layer is sufficiently linear over an extremely wide dose range (10 times the intended dose), then a judicious extrapolation is justified for a special application requiring a dose higher than tested before.

Many food products are regularly packaged in laminate pouches, made primarily with high-durability polymers, including PET. Some producers of laminate food packaging have extensive experience, well-established quality assurance procedures, and excellent records of regulatory approval of food-grade laminate products. Their procedures can be adapted to ensure retention of the lamination between the PET (or polyimide) layers and the aluminium inner-layer following high-dose irradiation.

7.6 Conclusions

Food packaging technology has made dramatic advances over the last 30 years in all the scientific and technological fields relevant to reliable and safe packaging for prepackaged precooked irradiated foods. The most important advances relate to:

- characterization of physical properties required to protect specific foods
- design of materials and packaging structure to meet specific requirements
- polymeric materials grades, barrier properties and cleanliness
- radiation durability of new polymers, exceeding 10^5 kGy for many of them
- analytical methods for testing of polymer properties and cleanliness
- safety and reliability assessment methodologies

On the basis of existing data and the insights gained from the above advances, the Study Group concluded that:

- The currently used trilaminate pouch developed by Natick with polyethylene as the food-contacting layer (which is approved) is of proven safety, based on experience and long-term wholesomeness testing.
- The concept of double packaging, which provides a single approved layer in contact with the food, overwrapped with a laminated package with the requisite physical properties, should be exploited.
- The concept of chemiclearance should be applied to packaging, since the relationship between polymer structure and resistance to radiation damage (including extractable products) can be established.
- Approving a particular packaging material for use in a radiation sterilization procedure arrived at on the basis of extrapolation above the currently used dose is also possible. It can be done straightforwardly by referencing an established damage–dose response relationship and extrapolating the packaging durability assessment to the projected higher dose. If the extrapolated assessment indicates no compromise in safety or functionality, then the procedure can be considered acceptable.

8. Processing considerations

Processing food by irradiating to high doses is essentially identical to radiation processing of food to any dose up to the currently accepted limit of 10 kGy. However, the accepted and generalized concept of the hazard analysis critical control point (HACCP) system is that the potential hazards associated with a particular technology together with available critical control points should be reconsidered when modifying that technology – even for seemingly minor alterations. Modifications may include a change in established and accepted dose limits and the introduction of new process procedures or applications. The use of high radiation doses to process precooked and prepackaged high-moisture

foods represents a change in the objective as well as in the details of the process. The relevant process parameters include extended residence times of a frozen product in an irradiation facility and higher dose rates in order to reduce treatment times. As a consequence of changing such primary parameters, several other parameters are changed or adapted and need special consideration. Likewise, product handling may be affected by requirements for low temperatures (during pretreatment storage and radiation processing) and for durable barrier packaging. Accordingly, the Study Group reviewed irradiation and HACCP issues relevant to the radiation processing of foods in the dose range above 10 kGy.

8.1 Radiation sources

It is generally accepted and has been adopted as a Codex Alimentarius General Standard (2) that only the following radiation sources are suitable for radiation processing of food:

- radioisotope sources: cobalt-60 or caesium-137
- machine sources: electrons up to 10 MeV and X-rays from electrons up to 5 MeV.

The radiation processing industry, which applies this technology for medical sterilization, for paint and ink curing, and for initiating polymerization, has an exceptionally high record of occupational safety. This is due, among other reasons, to the fact that well-trained personnel operate the facilities, that the nature of the irradiated products requires a high level of quality assurance, that irradiation facilities have inherent safety features, and finally that standards and supervision by responsible authorities enforce adherence to good manufacturing procedures.

The radioisotope cobalt-60 is produced intentionally from metallic cobalt-59 which, when inserted into specifically designed nuclear power reactors, absorbs neutrons. The activated metal does not need any waste refinement treatment, and is doubly-encapsulated as rods or discs in stainless steel casings before being released to irradiation facilities. Even for very high specific activities, the unavoidable self-heating could not liquefy the solid metal. Cobalt-60 rods of very high specific activity can be configured in appropriate source frames for use in high-dose and high-dose-rate processing. The technology of cobalt-60 production, handling and use is well established worldwide.

The radioisotope caesium-137 is obtained from spent nuclear fuel elements, but is not readily available in the quantities that would be needed for commercial exploitation.

The quantum energies of the gamma-rays emitted from both of these acceptable radioactive sources, 0.66 MeV for caesium-137 and 1.13 and

1.33 MeV for cobalt-60, are well below the thresholds for photonuclear activation of any chemical element. Consequently, even at the highest imaginable doses, no radioactivity can be induced in the exposed food by these sources.

As indicated above, electrons from machine sources are limited in energy to 10 MeV, and primary electrons for producing X-rays are limited in energy to 5 MeV. For the production of X-rays, converters are used that consist of a material of high atomic number for better efficiency in energy conversion and that have good physical properties such as a high melting point; tantalum and tungsten are the most frequently used materials. Such materials, appropriately cooled, can withstand the high electron beam power needed for X-ray applications.

The possibility that radioactivity might be induced in food processed by electrons or X-rays needs to be considered (*1*). The most important physical processes to be taken into account are: the excitation of isomeric states in nuclei by high energy photons; photonuclear reactions; and the capture of neutrons produced in photonuclear reactions (principally from deuterium). With respect to induced activity, estimates show that only irradiation by X-rays is of concern, since the activity produced by 10-MeV electron irradiation is significantly lower than that produced by 5-MeV X-ray irradiation for equal absorbed doses. In the latter case, neutrons are produced in the food by photonuclear reactions. After “thermalization”, they are captured by certain elements in food yielding extremely small amounts of short-lived radionuclides. Since the energy of the X-rays is limited to 5 MeV, which is below the thresholds of photonuclear reactions in heavy metals such as tungsten and tantalum, no neutrons are emitted from the converter target.

The significance of induced activity in food resulting from high-dose irradiation can be assessed by comparing it to the concentration of naturally occurring radionuclides in the food (the most prevalent of which is potassium-40) and the internal body doses resulting from ingestion. Very conservative calculations show that the consumption of food irradiated to doses up to 100 kGy results in doses to the consumer that are at least a factor of 1000 below those from natural activity inherent in the human body, in food, and in the environment. The radiological impact of consumption of food irradiated to high doses would therefore be insignificant.

8.2 Dosimetry

Radiation dosimetry is a well-established technology that can be used over a wide dose range and in any anticipated application. Available ASTM standards, national regulations and certification laboratories

bear witness to the applicability of this standardized measuring technology (467, 468). It is based on distinct scientific principles described in several textbooks and monographs that are widely available (469–471).

The principles of dosimetry in general and of its application to food irradiation in particular are well established (472–478). There are four levels of dosimetry: absolute; reference; routine; and indicator. *Absolute* dosimetry systems are usually operated by metrological institutions and serve the purpose of certifying the physical quantity “absorbed energy dose” and its unit the gray (Gy) with very high accuracy and precision; such efforts are usually coordinated on an international level. The inconvenience of carrying out the required procedures limits their application in industrial radiation processing. Consequently, *reference* dosimetry systems are used and are calibrated against some absolute standard and then linked to the *routine* dosimetry system used in process control. In this way, dose measurements are traceable to national and international standards. Recently, label dosimeters have become available. Such systems change colour or exhibit changes in other easy-to-recognize features after reaching a certain dose level; they are useful in routine dosimetry. *Indicators* must not be confused with label dosimeters; what they have in common is that both are attached to the surface of the products. Indicators cannot “indicate” a dose value; their usefulness is in indicating that the products emerging from the irradiation have been treated.

The challenge of dosimetry for food irradiation is the wide dynamic dose range associated with diverse applications, the dose ranging from a minimum of 10 Gy to more than 50 kGy and the applications ranging from sprout inhibition to insect disinfestation, food sterilization and product modification.

A range of four orders of magnitude is often not a problem for many metrological technologies; however, most established dosimeters are specific to a particular, narrower dose range. This limitation is especially true for dosimeters suitable for routine applications in food irradiation. Consequently, in order for an irradiation facility to provide services for the entire dose range, several dosimeter systems covering overlapping dose ranges must be used. Commercial contractors already provide such services covering any dose range, and they have at hand several dosimetry systems to prove that the dose received complies with customer or regulatory requirements.

Most dosimetry systems, especially routine dosimeters, are sensitive to dose rate, in particular to the dose rates of 10^6 – 10^8 Gy/s that are associated with electron beam processing facilities. In this connection, it

must be recognized that several radiation effects are also dose-rate dependent, such as the loss of certain micronutrients, so dosimeters suitable for these ranges of dose rates must be used.

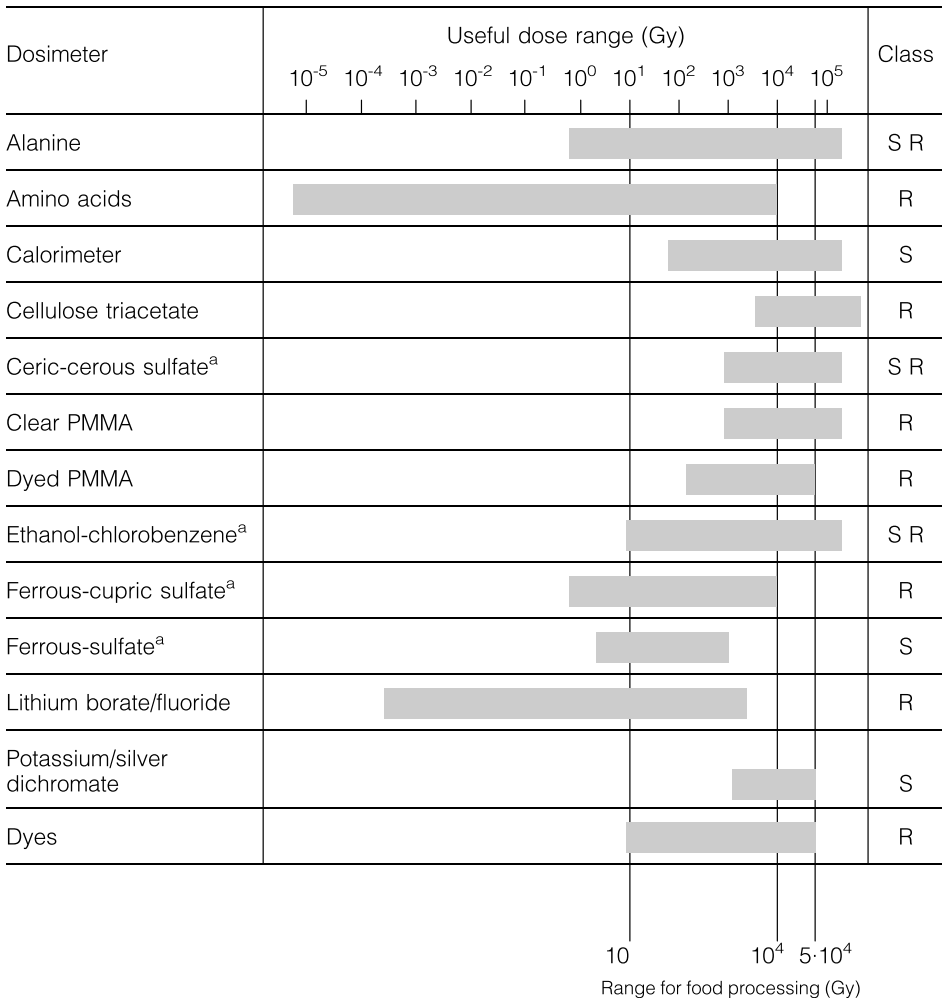
Accordingly, dosimetry and process control – including setting of the target dose – must take into consideration these and other chemical and physical effects (479–487). Most dosimeters, for example, are affected by temperature and phase during radiation processing (e.g. the radiation chemistry of liquid and frozen aqueous solutions is completely different); consequently, it must be carefully established that the chosen dosimeter maintains its metrological characteristics at the specified processing temperatures. There are other environmental factors affecting dosimeter performance, including humidity; however, in most instances, shielding the dosimeter against humidity by enclosing it in a plastic film would be sufficient to avoid any problem. It must also be recognized that dosimeters are sensitive to temperature during readout; however, in most cases, simple correction functions apply.

Once the appropriate dosimeter or dosimeters have been chosen, it is typically only necessary to map the dose distribution within a product or product model and to couple that information with the measured time the product remains in the irradiation treatment cell (dwell time) for either continuous or batch operation, in order to obtain the corresponding dose rates. The doses and dwell times used for determining dose rate can be less than for the actual processing, since the operator ultimately relies upon timers and conveyor speed controllers to deliver the desired dose to the product. In this way, the operator ensures that the effect on the dosimeter remains within its working range. Final verification of the dose and dose spread can be made using a dosimeter suitable for the intended range. Various solid state systems (e.g. alanine powders and radiochromic films), solid or liquid calorimeters, and electronic (i.e. charge integrating) devices are available for high-dose operations and can be used as routine secondary dosimeters; they can be referred back to primary standards for certification. All of these approaches have been used successfully in achieving doses of 30–75 kGy both in radioisotope and machine source facilities (Table 40).

8.3 Process control

Food irradiation is a self-limiting process. A dose that is too low would not achieve the intended purpose, prompting the customer to challenge the service provider for not providing the contracted dose and not achieving the desired effect. A dose that is too high would affect the sensory quality of the product, again prompting the customer to challenge the operator for exceeding the contracted dose and spoiling the

Table 40
Examples of routine and reference standard dosimeters



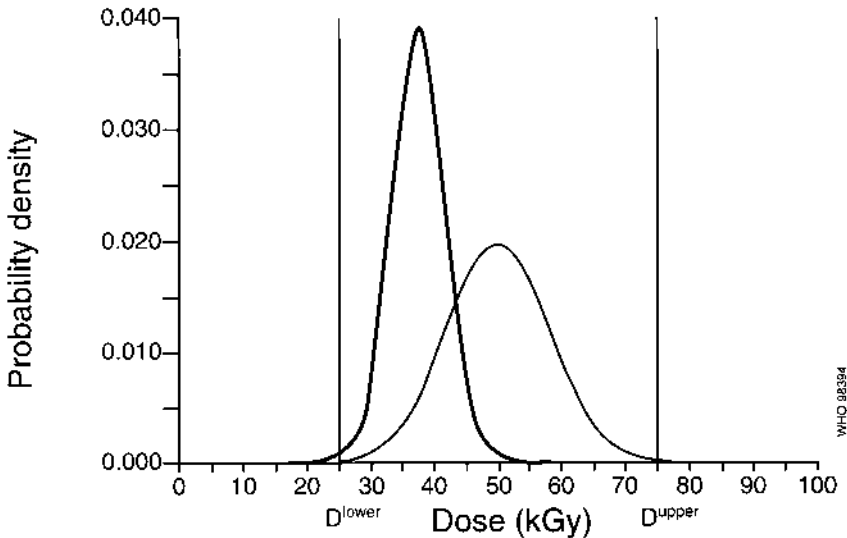
PMMA = polymethylmethacrylate; R = routine; S = reference standard.
^aAqueous solution

product quality. Process control is the methodology used to achieve the twin goals of a dose above the minimum required for sterilization and below the maximum imposed by product quality. It also helps to settle disputes between contractor and either regulatory officials or customers and it produces records for later examination and verification (488).

In practice, process control relies primarily on past research and practical experiences regarding the effectiveness of the radiation treatment, i.e. the minimum dose necessary to achieve the beneficial effect and – if applicable – the maximum dose tolerable to avoid any

Figure 21

Product dose distributions for two radiation treatments within lower (D^{lower}) and upper (D^{upper}) process limits for absorbed dose



The narrower curve shows the dose distribution that would be chosen for certain products and their quality considerations. Irradiation facility parameters are set so that less than 0.1% of the material to be irradiated receives a dose of less than D^{lower} for effective treatment in both cases; less than 0.1% receives a dose greater than D^{upper} for the wider dose distribution (target minimum dose arbitrarily chosen at 25 kGy)

detrimental effects to the product. In high-dose radiation processing, where the aim is to achieve sterility, factors to be taken into account in determining the minimum effective dose include the expected microbial load, the known radiation sensitivity of the relevant species of microorganisms, and the required reduction factor for that species. Usually the “12D-concept” is applied (see section 5). The required reduction (10^{12}) in the population of the most resistant spores of *Clostridium* spp. translates into a treatment in various foods of 24–42 kGy. In order to guarantee an effective treatment, the operator sets the target minimum dose at a safe level above this minimum value (Fig. 21: lower process limit D^{lower} = minimum effective dose). From sets of dosimeters placed in replicate at the expected minimum dose positions, a statistical distribution is obtained that is characterized by mean value and standard deviation. For quality control procedures, a lower alert limit is used to keep any fluctuations of the dose at the expected position of the minimum dose well above the process limit. The same considerations apply, but inversely, for any upper dose limit (Fig. 21: upper process limit D^{upper} = maximum tolerable dose). This approach

will result in a dose distribution within the irradiated food lot, batch or consignment that clearly falls between the targeted dose limits (Fig. 21). Depending on technical conditions and customer requirements, the resulting dose distribution could either exploit the maximum tolerable value in setting the ratio between maximum and minimum doses or be restricted to a very narrow dose spread.

It should be recognized that the useful dose range in sterilization applications (the range between maximum and minimum doses) will, in many instances, be less than that used in commercial irradiation facilities where the ratio between maximum and minimum dose is less than 3 but often more than 2 (1). For comparison, in the Raltech study (489), the dose distribution was characterized by a mean dose of 59 kGy, a minimum dose of 47 kGy, and a maximum dose of 71 kGy; the D_{\max}/D_{\min} ratio was 1.5. This narrower dose spread is achieved by less loading of the carriers (thus limiting the throughput), but assures a safe and acceptable product. It should be emphasized that none of the over 300 000 samples prepared for the Raltech study swelled or was otherwise spoiled.

The most anticipated application of high-dose irradiation of food is to achieve sterility to make the product stable at room temperature. Process control is very critical for delivering the minimum dose required to achieve the desired effect. Unlike medical disposables, the available margin for the maximum tolerable dose might be very narrow for a food whose quality attributes are sensitive to excessively high doses; its flavour, texture and appearance might be compromised. Consequently, the dose distribution in each and every lot being irradiated must be consistent with the technological limits appropriate to that product, and process control procedures must be introduced to keep the dose within the appropriate lower and upper margins.

Good irradiation practice requires that a technically practical narrow range between D_{\min} and D_{\max} be targeted. Specific requirements for radiation-sterilized products can best be fulfilled in this way, using existing radiation processing facilities.

Besides good irradiation practice, the general rules of good manufacturing practice should also be followed rigorously. In particular, the initial microbial load of the product must be kept as low as possible (490) and, therefore, high standards of hygiene in product preparation and handling are required.

From a regulatory standpoint, lower and upper legal dose limits could be set – if appropriate – that accommodate the lower and upper process dose limits. For example, it could be argued that legal limits are inappropriate for sprout inhibition of potatoes: if the process fails

because too low a dose was used, there is no health hazard to the consumer; if the dose used was far too high, the quality of the potatoes is impaired, but again no health hazard would be posed by consuming such potatoes. However, it may be appropriate to set limits when irradiating for specific purposes, e.g. irradiating chicken parts to eliminate pathogenic microorganisms. Consequently, United States regulations currently require a minimum dose of 1.5 kGy in order to fulfil the purpose of the radiation treatment. It is reasonable for authorities to set the lower legal dose limit in high-dose application at or above the lower process limit that had been determined and validated as necessary to sterilize a particular product. Again, however, too high a dose in sterilization treatments could impair the product's quality, but without any toxicological consequences, and would therefore limit its suitability for consumption — so again no legal limits need to be specified.

Process control in radiation processing relies on controlling the minimum and maximum doses throughout a given consignment, batch or treatment. In high-dose processing, the main purpose is to achieve microbial sterility, which to satisfy the 12D-concept requires a minimum dose, depending on the food, of 24–42 kGy. This requirement is met by setting process parameters in such a way that the resulting dose distribution at the expected position of the minimum dose is well above the specified minimum dose (Fig. 21). This minimum is achieved by choosing a tolerable error probability and calculating a tolerance range from the standard deviation of the respective measurements and the tolerance factor determined by number of measurements and applicable error probability. For example, a tolerable error probability of 0.1% would have a tolerance range of about 3.1. It is generally accepted (1, 2) that the maximum dose for any treatment under commercial circumstances is about 50% above the average dose and that the ratio between maximum and minimum dose can be kept to less than 3.0; the average dose can be expected to be the mean of the maximum and minimum doses.

With regard to regulatory limitations, there tends to be a misunderstanding of the term “overall average dose”. This misunderstanding is evident in connection with its numerical value of 10 kGy for low-dose applications adopted by the Joint Expert Committee in 1980 (1) and accepted by Codex Alimentarius in 1983 (2). Some regulations specify values lower than 10 kGy for the overall average dose for certain groups of food in order to avoid doses in any part of the food being greater than 10 kGy. The meaning of this quantity can best be understood by bearing in mind that toxicological potential is linked to chemical change and that the formation of radiolysis products is linearly proportional to dose (see section 3) in the dose range of interest (~100 kGy). This linearity implies

that any “over-treatment” is compensated by “under-treatment”, so only the average formation of radiolysis products is relevant. Consequently, overall average dose denotes a grand mean of doses applied to the food; regulatory limitations – if needed – can be derived in the light of Fig. 21 and the discussion above.

8.4 Environmental parameter control

Food processing in general requires control not only of process parameters but also of a range of environmental parameters. The basic approach is to isolate the food from the environment by enclosing it within packaging material that provides a barrier to molecular transmission and, in some cases, to light penetration. Since the main intended effect of radiation processing of food to high doses is the elimination of pathogenic and spoilage microorganisms and, hence, the achievement of sterility, the packaging material must prevent any recontamination by ubiquitous microorganisms in the environment, yet not introduce any undesirable effects as a consequence of the irradiation (see section 7).

With the exception of product improvement applications, such as increased juice extraction, high-dose radiation processing will always be primarily part of a “combination treatment” directed towards producing a shelf-stable product. The combination involves: heating to inactivate proteolytic enzymes, which are rather radiation-insensitive and not completely inactivated by even the radiation doses considered at present; vacuum packing to eliminate oxygen and to retain volatile flavorants; and freezing the product and maintaining the frozen state during irradiation, which is most important for minimizing side effects such as the formation of off-flavours. Since the product is stored deep-frozen prior to irradiation, it must be handled as other deep-frozen foods in order to avoid “melt–thaw–freeze” damages and “freezer-burn” caused by recrystallization. Packaging and packing conditions can be selected that permit the use of a “controlled atmosphere” or a “modified atmosphere”, which offers the potential of achieving product stabilization with lower doses.

A radiation-sterilized product, heat-treated for enzyme inactivation (which corresponds to being precooked and ready-to-eat), packaged to shield the product from recontamination, and sealed under vacuum in order to suppress oxidative processes, should be very insensitive to post-irradiation environmental factors, in particular humidity and temperature. It should remain stable and retain its qualities for as long as the package retains its integrity (see Annex 1). Radiation-sterilized diets have been used in hospitals in Scotland (491) and Washington, United

States (492); however, this application was discontinued for practical reasons when more stringent regulations hampered the further use of such products at one hospital and an irradiation facility was no longer available at the other. Samples of irradiated ham prepared for the 1977 joint Apollo–Soyuz space flight and kept at ambient conditions are still intact 20 years later in their original multilayered flexible packages. Since the 1980s, irradiated foods, including bread, breakfast rolls, beefsteak, ham and smoked turkey, have been used regularly on space flights (493). South Africa has a conditional clearance for sterile meats for use in producing ready-to-heat-and-eat meals for use by outdoor enthusiasts. Such products are hermetically sealed in mechanically-resistant, oxygen-impermeable, light-shielding plastic pouches that can withstand extreme environmental conditions and exhibit prolonged shelf-life. Several other applications are anticipated, and there are publications in scientific literature describing the sensory and nutritional quality of radiation-sterilized foods (125, 251, 304, 494, 495).

8.5 Re-irradiation

Under certain circumstances, it might be justified to process a food commodity that has already been irradiated (1). Examples of such situations include: dry products, such as grain, irradiated for insect disinfestation where re-infestation requires repetition of the treatment, as with fumigation; products manufactured from a raw material that had been irradiated for some purpose, such as onions previously irradiated to inhibit sprouting or dried onions prepared from irradiated onions, but where the final product needs to be processed by ionizing radiation for some justified purpose; and an irradiated minor ingredient, such as spices, where the final product containing this ingredient is to be irradiated for a justified purpose. It was concluded that the additional amount of radiolysis compounds in the final products would be insignificant and, hence, that this practice would be acceptable (1, 2, 490). This rationale also applies for products, processed to higher doses by ionizing radiation in order to obtain sterility, that contain irradiated ingredients or raw materials; an example is a composite food or meal containing vegetables, meats and spices, all previously irradiated for purposes other than sterilization. The dose and incremental amount of radiolysis compounds would be insignificant and, hence, the use of previously irradiated ingredients in products to be sterilized by irradiation would not need special processing consideration. Situations other than these that require repeated irradiation are not in compliance with good manufacturing practice and should be considered unacceptable. It should be noted that fractionated irradiation – where the full dose is applied in two or more

instalments — is *not* considered to be a repeated irradiation; fractionation could occur when the irradiation is interrupted for technical reasons (e.g. failure of the transport system).

8.6 Conclusions

In view of these considerations, the Study Group concluded that:

- the minimum absorbed dose needed to sterilize a food product can be accurately and reproducibly measured by following standardized dosimetric procedures;
- the ratio of maximum to minimum absorbed dose in any processed food lot, batch or consignment can be accurately and reproducibly defined from the dosimetric measurements;
- the processing and environmental parameters essential for ensuring that the food product is sterilized within the targeted dose range under technologically prescribed conditions can be properly monitored and recorded; and
- the overall handling of the product and process can be sufficiently controlled to ensure that products receive the required sterilizing dose, either in one treatment or in a properly fractionated sequence of treatments, and that they are not re-irradiated unless technically justified.

9. Conclusions

9.1 Wholesomeness: safety and nutritional adequacy

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. This conclusion is based on extensive scientific evidence that this preservation process can be used effectively to eliminate spores of proteolytic strains of *Clostridium botulinum* and all spoilage microorganisms, that it does not compromise the nutritional value of the foods, and that it does not result in any toxicological hazard. Recognizing that, in practice, the doses applied to eliminate the biological hazards would be below those doses that might compromise sensory quality, the Study Group concluded that no upper dose limit need be imposed. Accordingly, irradiated foods are deemed wholesome throughout the technologically useful dose range from below 10 kGy to envisioned doses above 10 kGy.

9.2 Substantial equivalence

In assessing risk, the Study Group concluded that irradiation to high doses is essentially analogous to conventional thermal processing, such as the

canning of low-acid foods, in that it eliminates biological hazards (i.e. pathogenic and spoilage microorganisms) from food materials intended for human consumption, but does not result in the formation of physical or chemical entities that could constitute a hazard. Abundant and convincing data indicate that high-dose irradiated foods do not contain either measurable levels of induced radioactivity or significant levels of any radiolysis products distinct from those found in unirradiated foods. The theoretical maximum levels that might be formed would be so low as to be of no toxicological consequence. Accordingly, none of the toxicological data derived from extensive animal feeding studies reveals any teratogenic, carcinogenic, mutagenic or other harmful effects that are ascribable to high-dose irradiated foods. For these reasons, the application of “risk assessment” in the currently accepted sense¹ is not appropriate to the toxicological assessment of foods preserved by high-dose irradiation. In this context, the concept of “substantial equivalence” may be more appropriate. High-dose irradiated foods are indeed as safe as food materials sterilized by thermal processing, which humans have been eating for more than a century.

9.3 Applications

The Study Group concluded that high-dose irradiation, conducted in accordance with good manufacturing practices and good irradiation practices, could be applied to several types of foods to improve their hygienic quality, to make them shelf-stable, and to produce special products. These foods are envisaged to include, but not be limited to: spices and other dry food ingredients; prepackaged precooked foods that could be stored at ambient temperature for extended periods; and sterilized meals for specific target groups (such as disaster victims, outdoor enthusiasts, and the immunocompromised). Components of all classes of foods whose sensory qualities are not compromised could be irradiated to high doses, either singly or in any combination. Packaging materials that are technically applicable and approved should be used as appropriate.

9.4 Global standardization

The Study Group concluded that appropriate steps need to be taken to establish the technological guidelines implied by these conclusions and to communicate them through Codex Alimentarius standards.

¹ In 1997, the Codex Alimentarius Commission adopted, on an interim basis, the following definition for risk assessment: “A scientifically based process consisting of the following steps: (i) hazard identification; (ii) hazard characterization; (iii) exposure assessment; (iv) risk characterization.”

10. Recommendations

1. The substantial benefit to food safety and food availability that would accrue directly from the broad application of food irradiation requires that steps be taken to put this technology into wider practice. These steps will involve standardization, communication and education.
2. WHO, in collaboration with FAO and IAEA, should:
 - coordinate the preparation of documentation and the drafting of appropriate technical language for adoption of standards by the Codex Alimentarius Commission;
 - prepare appropriate brochures and documents that integrate food irradiation into existing guidelines and rules governing the safe production, distribution and handling of food in order to minimize the spread of microbiological contamination and incidence of foodborne illnesses;
 - organize and participate in appropriate training courses and workshops to educate food regulators and food workers about the role food irradiation could, and should, play as a control measure in the framework of the application of the hazard analysis critical control point (HACCP) system.
3. WHO should take the lead in advising international agencies and national ministries of health on the implementation of integrated strategies, including food irradiation, for preventing the transnational spread of pathogens in human food and animal feed, for controlling foodborne illnesses, and for enhancing the availability of safe and nutritious foods.

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Annex 1

High-dose irradiated foods – practical experience

Introduction

Practical experience in the use of high-dose irradiated foods demonstrates that the important quality attributes of such foods are retained during processing and subsequent storage and confirms that the target average doses used to eliminate any microbiological hazard are effective and sufficient. Early researchers developing these products relied on the evaluations made by technical panels and small consumer panels to refine product formulation and processing, but they had no significant opportunity to validate the laboratory results with large numbers of consumers. Regulatory limitations worldwide have precluded any such large-scale testing, so feedback on the acceptability of the high-dose irradiated foods could only be obtained where special niches of approved use existed. Such special groups for which approval was obtained include: immunocompromised hospital patients; United States and Russian astronauts; and military personnel and outdoor enthusiasts in South Africa.

High-dose irradiated products

Persons with compromised immune systems

Beginning in 1974, the Fred Hutchinson Research Center in Seattle, Washington, offered radiation-sterilized food items to patients with compromised immune systems so as to maintain their nutritional health and to prevent ingestion of foodborne infective agents (1). Food not suitable for autoclaving to destroy microbial contaminants, such as breads, pancakes, tortillas, crackers, stuffing mix, pastries, cereals, dry beverages, snacks and candies, nutritional supplements, meats and condiments, were irradiated to achieve sterility. These products were very favourably received by the patients. Because the irradiation source at the University of Washington had decayed to too low a level of activity, the use of irradiation was discontinued in 1988. The additional staff time needed to prepare the extensive documentation required for upgrading the source and for renewing approval for its use was considered prohibitive (S.N. Aker, personal communication).

Deep-frozen meals irradiated to an average dose of 75 kGy have also been produced in the Netherlands for use by hospital patients.

Programmes to inform high-risk individuals about the advantages of irradiation are under way in the United States. As part of the food safety initiative, Iowa State University has developed video-based educational

materials on food irradiation technologies and microbial food safety. These materials are intended for use by health care and other professionals when working with immunocompromised persons and their care givers.

Astronauts

From the inception of the United States space programme, astronauts have consumed irradiated food while in space. Irradiated products were eaten by United States astronauts on the Apollo 17 flight to the moon in December 1972 and on joint space flights with Soviet cosmonauts. Between 1981 and 1986, 228 portions of irradiated meat products and 121 bakery products were consumed (2). These irradiated foods were considered highly acceptable (3) and were selected because of their higher sensory quality compared to thermally-processed counterparts (2). Irradiated beefsteaks were also used on NASA Space Shuttle flights in 1993 (4). In addition to steaks, the United States Army Natick Research, Development and Engineering Center produced irradiated smoked turkey slices, corned beef, chicken, burritos, pork chops and pizza for the shuttle flights. In 1996, over 2500 beefsteaks, sliced turkey and other meat products were produced and radiation-processed (5). Four new products for NASA were also developed. In sensory panel evaluations, the new irradiated grilled beefsteak, breaded chicken breast, pork chops and corned beef all received scores above that required for shuttle foods.

South Africa

South Africa markets irradiated shelf-stable foods and a variety of other irradiated products including spices and herbs, honey products, torulite yeast, garlic, egg products and fresh vegetables. The type of shelf-stable foods and consumer response to them are of particular interest.

Research into irradiated shelf-stable meat products was initiated during 1977 as a result of a request from the Armed Forces. During the early 1980s, several products were developed in conjunction with food scientists from the Council for Scientific and Industrial Research and from Technicon of Pretoria. Researchers irradiated novel convenience foods that cannot be satisfactorily prepared by alternative methods such as canning or retorting (6); 12 dishes were tested, including grilled chicken, curried chicken, bacon, curried beef and a Malaysian dish called bobotie. Between 1982 and 1987, approximately 20 000 portions were produced and evaluated by individuals, expeditions and the Armed Forces. In 1989, approval was obtained from the Department of Health to supply shelf-stable irradiated food to the Armed Forces.

Table A1

Sale in portions (~150 g) of shelf-stable irradiated meat in South Africa

Year	Military	Non-military
1987	18 660	NA
1988	20 000	NA
1989	25 000	2 859
1990	25 000	5 726
1991	415 750	8 286
1992	415 750	9 870
1993	415 750	12 826
1994	236 650	11 867
1995	206 590	22 355
1996	0	25 579
1997	0	37 147

Reproduced from Bruyn (7) with permission.

In 1987 and 1988, approximately 20 000 portions of shelf-stable meat items were sold to the military (7). The quantity increased to 25 000 per year in 1989 and 1990, then to over 400 000 per year in the period 1991–1993 (Table A1). Annual sales decreased to over 200 000 in 1994 and 1995 owing to restructuring of the military.

The total of about 1.8 million portions (about 3 million kg) of high-dose irradiated foods consumed by the military provides a basis for assessing acceptance by users. Many special forces personnel have relied upon these ration portions for their entire intake of protein for extended periods. There have been no incidences of adverse health responses reported (7). Moreover, these foods were found to be “consistently of the highest quality.”

In 1989, the sale of shelf-stable meat items to non-military customers began with 2859 portions sold in the first year, increasing to almost 10 000 in 1992 and 25 579 in 1996. Increased sales after 1989 were associated with permission being obtained to sell in selected hiking and outdoor shops and to undertake a marketing programme, which included tasting. High-dose irradiated products were clearly popular among yachtsmen and other outdoor enthusiasts, based on feedback from them. Yachtsmen competing in a race from the Cape to Rio accounted for a large quantity sold in 1996.

A marketing survey among the general population found that, while only 15% initially indicated they were likely to purchase irradiated food, the proportion willing to purchase increased to 54% after receiving visual information. It also found that, after receiving information *and* tasting the food, 76% indicated they would purchase the irradiated shelf-stable product, while only 5% said they probably would not.

Conclusions

The acceptability in niche markets of various high-dose irradiated products, including meat items and whole meals, and the lack of any health problems resulting from their consumption, provide practical evidence of the effectiveness and appropriateness of the radiation-sterilization process.

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* Prices in developing countries are 70% of those listed here.