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

Report of a Joint FAO/IAEA/WHO Study Group

World Health Organization
Geneva 1999
8. Processing considerations  150
  8.1 Radiation sources  151
  8.2 Dosimetry  152
  8.3 Process control  154
  8.4 Environmental parameter control  159
  8.5 Re-irradiation  160
  8.6 Conclusions  161

9. Conclusions  161
  9.1 Wholesomeness: safety and nutritional adequacy  161
  9.2 Substantial equivalence  161
  9.3 Applications  162
  9.4 Global standardization  162

10. Recommendations  163

Acknowledgements  163

References  164

Annex 1
High-dose irradiated foods—practical experience  194
Joint FAO/IAEA/WHO Study Group on High-Dose Irradiation (Wholesomeness of Food Irradiated with Doses above 10 kGy)


Members

Professor R. V. Bhat, Deputy Director, National Institute of Nutrition, Indian Council of Medical Research, Jamai-Osmania, Hyderabad, India (Vice-Chairman)

Professor G. W. Gould, Bedford, England

Dr R. Gross, German Agency for Technical Cooperation, Eschborn, Germany

Professor P. C. Kesavan, Director, Bioscience Group, Bhabha Atomic Research Centre, Trombay, Bombay, India

Dr E. Kristiansen, Department of Pathology, Institute of Toxicology, Danish Veterinary and Food Administration, Ministry of Food, Agriculture and Fisheries, Søborg, Denmark

Dr A. O. Lustre, Director, Food Development Centre, National Food Authority, Manila, Philippines

Dr K. M. Morehouse, Office of Premarket Approval, Centre for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, DC, USA (Rapporteur)

Professor G. E. Osuide, Director-General, National Agency for Food and Drug Administration and Control, Lagos, Nigeria

Dr T. A. Roberts, Reading, England (Chairman)

Dr O. Sapora, Laboratory of Comparative Toxicology and Ecotoxicology, Istituto Superiore di Sanità, Rome, Italy

Dr H.-P. Weise, Federal Institute for Materials Research and Testing, Berlin, Germany

Secretariat

Dr A. Brynjolfsson, Wayland, MA, USA (Temporary Adviser)

Professor J. F. Diehl, Karlsruhe, Germany (Temporary Adviser)

Mr D. A. E. Ehlermann, Director and Professor, Federal Research Centre for Nutrition, Institute of Process Engineering, Karlsruhe, Germany (Temporary Adviser)

Professor J. Farkas, Department of Refrigeration and Livestock Products Technology, University of Horticulture and Food Industry, Budapest, Hungary (Temporary Adviser)

Dr Y. Haruvy, Advanced Projects Initiation, Division of Applied Radiation Technology, Soreq, Yavne, Israel (Temporary Adviser)

Dr F. K. Käferstein, Director, Programme of Food Safety and Food Aid, WHO, Geneva, Switzerland (Joint Secretary)

Mr P. Loaharanu, Head, Food Preservation Section, Secretariat of the International Consultative Group on Food Irradiation, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, IAEA, Vienna, Austria (Joint Secretary)
Dr G. G. Moy, Programme of Food Safety and Food Aid, WHO, Geneva, Switzerland
Professor J. Raffi, Director, Laboratory of Research on the Quality of Food, St Jerome
Faculty of Sciences, Marseilles, France (*Temporary Adviser*)
Mr M. Satin, Chief, Agro-Industries and Post-Harvest Management Service,
Agricultural Support Systems Division, FAO, Rome, Italy (*Joint Secretary*)
Dr C. A. Takeguchi, Phoenix Regulatory Associates, Ltd, Sterling, VA, USA
(*Temporary Adviser*)
Dr I. A. Taub, Senior Research Scientist, Sustainability Directorate, US Army Natick
Research, Development and Engineering Centre, Natick, MA, USA (*Temporary Adviser*)
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 kGy\(^1\) 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 ($D_{\text{max}}/D_{\text{min}}$) 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 ($D_{\text{max}}$) for this purpose.

Still higher doses are required for radiation sterilization of food, for instance, for immunos-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 (II).

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, $e_-$, 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$^+$) and hydroxyl radicals (OH$^-$); 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 (H₃O⁺) and OH⁻. Various recombination and cross-combination reactions of the primary radicals are possible, the combination of H⁺ and OH⁻ regenerating water (H₂O). 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 × 10⁻⁴.) For neutral water subjected to the kinds of radiation permitted for use with food, the relevant G-values (using the older definition) for e, OH⁻, H⁺, molecular hydrogen (H₂) and hydrogen peroxide (H₂O₂) are 2.7, 2.7, 0.6, 0.4 and 0.7, respectively (18, 21). They indicate the predominance of e and OH⁻ 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 \( e^- \) or \( \text{OH}^\cdot \) 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 (\( \text{O}_2 \)) in the head space can influence the chemistry by introducing new pathways for reaction. Because of its high electron affinity, \( \text{O}_2 \) reacts readily with \( e^- \) and \( \text{H}^\cdot \) and with organic radicals. Reaction with the former leads to the formation of \( \text{O}_2^{2-} \) or \( \text{HO}_2^\cdot \), which react primarily to yield \( \text{H}_2\text{O}_2 \). Reaction with organic radicals leads to the formation of \( \text{RO}_2^\cdot \) radicals, which tend to react bimolecularly to form peroxides, but can also decompose unimolecularly to \( \text{R}^\cdot \) and \( \text{HO}_2^\cdot \). 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

---

\[ a \] 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− with solutes, including the nitrate ion; the histogram on the right corresponds to the oxidation of the ferrocyanide ion by OH•, whose yield is doubled by the reaction of e− with N2O. 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− 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− 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,

\[ 2\text{RCH}_2\text{C}^\circ\text{O} \rightarrow \text{RCH}_2\text{COCOCH}_2\text{R} \quad (1) \]
\[ \text{RCH}_2\text{C}^\circ\text{O} \rightarrow \text{RCH}_2 + \text{CO} \quad (2) \]

the likelihood of reaction (1) increases with a substantial increase in dose rate, since the concentration of RCH_2CO 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

* Reproduced from Taub et al. (28) with the permission of the publisher.
contribution of radicals corresponding to H\(^+\) 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\(^+\) and OH\(^+\) will differ from that initiated by the solvated electron, but some of the same intermediates will be formed (28). H\(^+\) 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\(^+\) 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\(^+\) is involved in such cross-linking and how cross-linked products can be found without the initially added OH\(^+\) (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,
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$^-$ 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$^+$ and OH$^-$ 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$^-$ would tend to reduce the G-values of products traceable to these precursors. Moreover, because the iron is capable of reacting intramolecurarily 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.

\[ \text{Signal intensity} \]

\[ \text{Magnetic field (gauss)} \]

---

\( ^a \) 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 \( \text{RCH}_2\text{O}(\text{C}=\text{O})\text{CH}_2(\text{CH}_2)_{15}\text{CH}_3 \). 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**

\[
\begin{align*}
\text{RCH}_2\text{O}(\text{C}=\text{O})\text{CH}_2(\text{CH}_2)_{15}\text{CH}_3 & \quad \text{e}^- \\
\text{RCH}_2\text{O}(\text{C}^\cdot)\text{CH}_2(\text{CH}_2)_{15}\text{CH}_3 & \quad \text{RC}^\cdot + \text{O}_2\text{CH}_2(\text{CH}_2)_{15}\text{CH}_3 \\
\text{RC}^\cdot + \text{O}_2\text{CH}_2(\text{CH}_2)_{15}\text{CH}_3 & \quad \text{RCH}_2\text{O}(\text{C}=\text{O})\text{CH}_2(\text{CH}_2)_{15}\text{CH}_3 \\
\text{RCH}_2\text{O}(\text{C}=\text{O})\text{CH}_2(\text{CH}_2)_{15}\text{CH}_3 & \quad \text{RCH}_2\text{O}(\text{C}=\text{O})\text{CH}_2(\text{CH}_2)_{15}\text{CH}_3 \\
\text{RCH}_2\text{O}(\text{C}=\text{O})\text{CH}_2(\text{CH}_2)_{15}\text{CH}_3 & \quad \text{RCH}_2\text{O}(\text{C}=\text{O})\text{CH}_2(\text{CH}_2)_{15}\text{CH}_3 + \text{RCH}_2\text{O}(\text{C}=\text{O})\text{CH}=	ext{CH}(\text{CH}_2)_{14}\text{CH}
\end{align*}
\]
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 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 propanediol esters (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-dodecyclohexanone (DCB), which can be derived from palmitic acid, has been detected in certain irradiated foods. The alkylcyclohexanones 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 μg 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 dextrins (47–49). It is also possible to calculate the yield of soluble dextrins 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 \( \ce{c^=} \), \( \ce{H^+} \), and \( \ce{OH^=} \) towards saccharides is very much like that towards alcohols and simple ethers. The \( \ce{c^=} \) has a low affinity for most of the groups except for the ether linkage and the carbonyl group. The \( \ce{OH^=} \) and \( \ce{H^+} \), 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:

\[
e_{s}^{-} + NO_{3}^{-} \rightarrow NO_{3}^{2-} \quad (3)
\]

\[
NO_{3}^{2-} + H^{+} \rightarrow NO_{2}^{-} + OH^{-} \quad (4)
\]

\[
2NO_{2} + H_{2}O \rightarrow NO_{2}^{-} + NO_{3}^{-} + 2H^{+} \quad (5)
\]

The overall stoichiometry is:

\[
2e_{s}^{-} + NO_{3}^{-} + H_{2}O \rightarrow NO_{2}^{-} + 2OH^{-} \quad (6)
\]

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' and OH'. It is reduced to an intermediate radical by e$_{s}^{-}$ and H' and is oxidized to the relatively stable tricarbonyl radical ion by OH' (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\textsuperscript{−}, H\textsuperscript{+} and OH\textsuperscript{−} because of its heterocyclic structure and prevalent carbonyl groups. However, it is the reaction with OH\textsuperscript{−} that ultimately leads to base damage and in part to single strand breakage in DNA. The OH\textsuperscript{−} 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\textsuperscript{−} 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.
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
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
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
Potential pathways for reaction of the alkyl (C_{17}) radical (from stearic acid): abstraction; combination to form the dimer; and disproportionation to form a double bond at a terminal carbon

a) \[ \text{CH}_3(\text{CH}_2)_{15}\text{C}^\cdot\text{H}_2 + \text{RCH}_2\text{O(C=O)}\text{CH}_2(\text{CH}_2)_{15}\text{CH}_3 \rightarrow \]
\[ \text{CH}_3(\text{CH}_2)_{15}\text{CH}_3 + \text{RCH}_2\text{O(C=O)}\text{C}^\cdot\text{H}(\text{CH}_2)_{15}\text{CH}_3 \]

b) \[ 2\text{CH}_3(\text{CH}_2)_{15}\text{C}^\cdot\text{H}_2 \rightarrow \text{CH}_3(\text{CH}_2)_{15}\text{CH}_2\text{CH}_2(\text{CH}_2)_{15}\text{CH}_3 \]

c) \[ 2\text{CH}_3(\text{CH}_2)_{15}\text{C}^\cdot\text{H}_2 \rightarrow \text{CH}_3(\text{CH}_2)_{15}\text{CH}_3 + \text{CH}_3(\text{CH}_2)_{14}\text{CH=CH}_2 \]

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 \(-30^\circ\text{C}\)\(^a\)

![Graph showing yield of hexane as a function of fat content.](image)

\(^a\) 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°C\(^a\)

\[ \text{Yield (µg/g} \times \text{fat per 10 kGy)} \]

Linoleic acid (%)

\[ 0.0 \quad 0.5 \quad 1.0 \quad 1.5 \quad 2.0 \]

\[ 0 \quad 5 \quad 10 \quad 15 \quad 20 \quad 25 \quad 30 \]

- Beef
- Chicken
- Pork

\(^a\) 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

\[ \text{Yield (ng/mg precursor)} \]

\[ 0 \quad 5 \quad 10 \quad 15 \quad 20 \quad 25 \]

\[ 0 \quad 2 \quad 4 \quad 6 \quad 8 \quad 10 \quad 12 \]

- Lean beef
- Fatty beef
- Pork
- Light chicken
- Dark chicken
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 propanedioldeister radicals will be formed. To emphasize the positional differences of the fatty acid moieties, these radicals will be denoted here as $\text{H}_2\text{C(O}_2\text{R''})\text{CH(O}_2\text{R')CH}_2$, showing the loss of $\text{O}_2\text{R}$ from the 1-position. Upon abstracting a hydrogen from other triglycerides, they become stable propanedioldeister products, $\text{H}_2\text{C(O}_2\text{R''})\text{CH(O}_2\text{R')CH}_3$. Accordingly, if scission is equally likely from the 1, 2, or 3 position in the glycerol backbone, then the yield of corresponding dioldeister isomers, namely $\text{H}_2\text{C(O}_2\text{R'')CH(O}_2\text{R')CH}_3$, $\text{H}_2\text{C(O}_2\text{R'')CH}_2\text{CH}_2\text{O}_2\text{R}$, or $\text{H}_3\text{CCH(O}_2\text{R')CH}_2\text{O}_2\text{R}$, will correlate with the level of precursor triglycerides containing the relevant $\text{O}_2\text{R''}$, $\text{O}_2\text{R'}$, and $\text{O}_2\text{R}$ fatty acids.

This correlation has been shown in a limited number of cases examined (29). Non-volatile propanedioldeisters were isolated from enzyme-inactivated chicken, beef, pork and ham samples irradiated to 30, 60 and 90 kGy. Propanediolpalmitate 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 propanediolpalmitate was 7.1% for POP and 5.3% for PPL, where O and L denote olate 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 propanediolpalmitate (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 propanediolpalmitate to its triglyceride precursors in four enzyme-inactivated muscle foods irradiated to 30, 60, and 90 kGy at \(-40^\circ\text{C}\)\(^b\)

\[\text{Dose-normalized yield (ug/g fat per 10 kGy)}\]

\[\text{Precursor abundance (%)}\]

- Ham
- Beef
- Chicken
- Pork

\(^a\) 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 propanediolpalmitate 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 conse-
quences 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 ±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 °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 sulphydryl, 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

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

Reproduced from Underdal et al. (79) with the permission of the publisher.
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 \((210 \text{ kGy for field beans, 180 kGy for lentils})\) 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
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 B_{12}, 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\(^a\)

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

\(^a\) Vitamin concentrations are given on a dry weight basis.
\(^b\) Significantly lower than frozen control.
\(^c\) Significantly higher than frozen control.
Adapted from Thayer (79) with the permission of the publisher.
The effect of radiation dose and temperature during irradiation on thiamine retention in pork

![Graphs showing thiamine fraction retained vs. dose for γ and e⁻ irradiation at different temperatures.](image)

© 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 watersoluble 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>
<thead>
<tr>
<th>Treatment</th>
<th>Radiation dose (kGy)</th>
<th>Thiamine (mg/100 g protein)</th>
<th>% loss</th>
<th>Thiamine (mg/100 g protein)</th>
<th>% loss</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Due to irradiation</td>
<td>Due to combined treatment</td>
<td>Due to frying</td>
<td>Due to irradiation</td>
</tr>
<tr>
<td>None (raw)</td>
<td>0</td>
<td>4.42</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>1.77</td>
<td>60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>0.95</td>
<td>78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>30.0</td>
<td>0.40</td>
<td>91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Irradiated, then fried</td>
<td>0</td>
<td>2.28</td>
<td>-</td>
<td>48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>0.76</td>
<td>-</td>
<td>83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>0.40</td>
<td>-</td>
<td>91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>30.0</td>
<td>0.07</td>
<td>-</td>
<td>98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fried, then irradiated</td>
<td>0</td>
<td>2.32</td>
<td>-</td>
<td>47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>2.02</td>
<td>13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>1.78</td>
<td>23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>30.0</td>
<td>1.49</td>
<td>36&lt;sup&gt;c&lt;/sup&gt;</td>
<td>66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> Compared to non-irradiated, non-fried samples.
<sup>b</sup> Compared to non-fried samples irradiated to the same dose.
<sup>c</sup> 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 10 kGy, 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'), hydroxyl radicals (OH') and solvated electrons (e⁻). The OH' radical is the most important; OH' 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 menstruum 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 \textit{Yersinia}, \textit{Pseudomonas}, \textit{Campylobacter}, \textit{Aeromonas} spp. and the vegetative cells of \textit{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. \textit{Escherichia coli} (including \textit{E. coli} O157:H7) and \textit{Arcobacter butzleri} are also quite radiation-sensitive, with \( D_{10} \)-values in the range 0.24–0.40 kGy in non-frozen products.
<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Product</th>
<th>Temp. (°C)</th>
<th>Atmosphere</th>
<th>D_{10} (kGy)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aeromonas</em></td>
<td>Ground fish</td>
<td>2±1</td>
<td>Air</td>
<td>0.140-0.193</td>
<td>149</td>
</tr>
<tr>
<td><em>hydrophila</em></td>
<td></td>
<td>22±1</td>
<td>Air</td>
<td>0.110-0.152</td>
<td>149</td>
</tr>
<tr>
<td><em>Arcobacter butzleri</em></td>
<td>Ground pork</td>
<td>NS</td>
<td>Vacuum</td>
<td>0.27±0.01</td>
<td>150</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>Roast beef</td>
<td>NS</td>
<td>Air</td>
<td>0.173±0.157</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>Gravy</td>
<td>NS</td>
<td>Air</td>
<td>0.181±0.167</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>Cauliflower (cooked)</td>
<td>NS</td>
<td>Air</td>
<td>0.207±0.099</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>Potato (cooked)</td>
<td>NS</td>
<td>Air</td>
<td>0.199±0.056</td>
<td>151</td>
</tr>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td>Ground pork</td>
<td>NS</td>
<td>Vacuum</td>
<td>0.19±0.01</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>Filet américain</td>
<td>18-20</td>
<td>Micro-aerophilic</td>
<td>0.08-0.11</td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>Ground beef</td>
<td>18-20</td>
<td>Micro-aerophilic</td>
<td>0.14-0.16</td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>Ground beef</td>
<td>0-5</td>
<td>Air</td>
<td>0.161</td>
<td>153</td>
</tr>
<tr>
<td></td>
<td>Ground beef (low fat)</td>
<td>30±10</td>
<td>Air</td>
<td>0.174</td>
<td>153</td>
</tr>
<tr>
<td></td>
<td>Ground beef (high fat)</td>
<td>4±1</td>
<td>Air</td>
<td>0.175</td>
<td>154</td>
</tr>
<tr>
<td></td>
<td>Ground turkey</td>
<td>0-5</td>
<td>Air</td>
<td>0.186</td>
<td>153</td>
</tr>
<tr>
<td></td>
<td>Ground turkey</td>
<td>30±10</td>
<td>Air</td>
<td>0.162</td>
<td>153</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>Minced pork</td>
<td>10</td>
<td>Air</td>
<td>0.826</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td>Minced pork</td>
<td>10</td>
<td>CO₂:N₂ (1:3)</td>
<td>0.750</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td>Roast beef</td>
<td>NS</td>
<td>Air</td>
<td>0.586±0.071</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>Gravy</td>
<td>NS</td>
<td>Air</td>
<td>0.411±0.059</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>Cauliflower (cooked)</td>
<td>NS</td>
<td>Air</td>
<td>0.528±0.065</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>Potato (cooked)</td>
<td>NS</td>
<td>Air</td>
<td>0.347±0.054</td>
<td>151</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Ground beef (low fat)</td>
<td>2</td>
<td>Air</td>
<td>0.36</td>
<td>156</td>
</tr>
<tr>
<td><em>E.coli O157:H7</em></td>
<td>Mech. deb. chicken</td>
<td>0</td>
<td>Air</td>
<td>0.26±0.01</td>
<td>157</td>
</tr>
<tr>
<td></td>
<td>Mech. deb. chicken</td>
<td>0</td>
<td>Vacuum</td>
<td>0.27±0.01</td>
<td>157</td>
</tr>
<tr>
<td></td>
<td>Ground beef</td>
<td>0</td>
<td>Vacuum</td>
<td>0.27±0.03</td>
<td>157</td>
</tr>
<tr>
<td></td>
<td>Ground beef (low fat)</td>
<td>4±1</td>
<td>Air</td>
<td>0.241</td>
<td>154</td>
</tr>
<tr>
<td></td>
<td>Ground beef (high fat)</td>
<td>4±1</td>
<td>Air</td>
<td>0.251</td>
<td>154</td>
</tr>
</tbody>
</table>
Table 6 (continued)

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Product</th>
<th>Temp. (°C)</th>
<th>Atmosphere</th>
<th>$D_{10}$ (kGy)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>Minced chicken meat</td>
<td>NS</td>
<td>Air</td>
<td>0.417-0.553</td>
<td>158</td>
</tr>
<tr>
<td></td>
<td>Mech. deb. chicken</td>
<td>2-4</td>
<td>Air</td>
<td>0.27-0.77</td>
<td>159</td>
</tr>
<tr>
<td></td>
<td>Minced pork</td>
<td>10</td>
<td>Air</td>
<td>0.573-0.648</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>Minced pork</td>
<td>10</td>
<td>CO₂:N₂ (1:3)</td>
<td>0.602-0.709</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>Roast beef</td>
<td>NS</td>
<td>Air</td>
<td>0.644±0.061</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td>Gravy</td>
<td>NS</td>
<td>Air</td>
<td>0.599±0.042</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td>Cauliflower (cooked)</td>
<td>NS</td>
<td>Air</td>
<td>0.564±0.055</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td>Potato (cooked)</td>
<td>NS</td>
<td>Air</td>
<td>0.532±0.047</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td>Ground beef (low fat)</td>
<td>4±1</td>
<td>Air</td>
<td>0.578-0.589</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>Ground beef (high fat)</td>
<td>4±1</td>
<td>Air</td>
<td>0.507-0.574</td>
<td>160</td>
</tr>
<tr>
<td><em>Salmonella anatum</em></td>
<td>Filet américain</td>
<td>18-20</td>
<td>Air</td>
<td>0.45</td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>Ground beef</td>
<td>18-20</td>
<td>Air</td>
<td>0.67</td>
<td>152</td>
</tr>
<tr>
<td><em>S. enteritidis</em></td>
<td>Ground beef (low fat)</td>
<td>2</td>
<td>Air</td>
<td>0.69</td>
<td>152</td>
</tr>
<tr>
<td><em>S. panama</em></td>
<td>Filet américain</td>
<td>18-20</td>
<td>Air</td>
<td>0.49</td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>Ground beef</td>
<td>18-20</td>
<td>Air</td>
<td>0.66</td>
<td>152</td>
</tr>
<tr>
<td><em>S. stanley</em></td>
<td>Filet américain</td>
<td>18-20</td>
<td>Air</td>
<td>0.61</td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>Ground beef</td>
<td>18-20</td>
<td>Air</td>
<td>0.78</td>
<td>152</td>
</tr>
<tr>
<td><em>S. typhimurium</em></td>
<td>Filet américain</td>
<td>18-20</td>
<td>Air</td>
<td>0.37</td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>Ground beef</td>
<td>18-20</td>
<td>Air</td>
<td>0.55</td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>Ground beef (low fat)</td>
<td>2</td>
<td>Air</td>
<td>0.59</td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>Minced pork</td>
<td>10</td>
<td>Air</td>
<td>0.403-0.860</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td>Minced pork</td>
<td>10</td>
<td>CO₂:N₂ (1:3)</td>
<td>0.394-0.921</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td>Roast beef</td>
<td>NS</td>
<td>Air</td>
<td>0.569±0.067</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>Gravy</td>
<td>NS</td>
<td>Air</td>
<td>0.416±0.058</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>Cauliflower (cooked)</td>
<td>NS</td>
<td>Air</td>
<td>0.590±0.075</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>Potato (cooked)</td>
<td>NS</td>
<td>Air</td>
<td>0.464±0.080</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>Mech. deb. chicken</td>
<td>20</td>
<td>Air</td>
<td>0.52-0.56</td>
<td>161</td>
</tr>
<tr>
<td></td>
<td>Mech. deb. chicken</td>
<td>20</td>
<td>Vacuum</td>
<td>0.52-0.56</td>
<td>161</td>
</tr>
<tr>
<td></td>
<td>Minced chicken</td>
<td>4</td>
<td>Air</td>
<td>0.436-0.502</td>
<td>162</td>
</tr>
<tr>
<td>Bacterium</td>
<td>Product</td>
<td>Temp. (°C)</td>
<td>Atmosphere</td>
<td>$D_{10}$ (kGy)</td>
<td>Reference</td>
</tr>
<tr>
<td>-------------------</td>
<td>-----------------</td>
<td>------------</td>
<td>------------</td>
<td>----------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>Minced chicken</td>
<td>4</td>
<td>$\text{CO}_2$</td>
<td>0.436–0.502</td>
<td>162</td>
</tr>
<tr>
<td></td>
<td>Minced chicken</td>
<td>4</td>
<td>$\text{N}_2$</td>
<td>0.550–0.662</td>
<td>162</td>
</tr>
<tr>
<td></td>
<td>Ground beef (low fat)</td>
<td>4±1</td>
<td>Air</td>
<td>0.621–0.624</td>
<td>154</td>
</tr>
<tr>
<td></td>
<td>Ground beef (high fat)</td>
<td>4±1</td>
<td>Air</td>
<td>0.618–0.661</td>
<td>154</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Roast beef</td>
<td>NS</td>
<td>Air</td>
<td>0.387±0.056</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>Gravy</td>
<td>NS</td>
<td>Air</td>
<td>0.360±0.043</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>Cauliflower (cooked)</td>
<td>NS</td>
<td>Air</td>
<td>0.427±0.055</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>Potato (cooked)</td>
<td>NS</td>
<td>Air</td>
<td>0.424±0.042</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>Mech. deb. chicken (in buffered peptone)</td>
<td>0</td>
<td>Vacuum</td>
<td>0.26–0.36</td>
<td>157</td>
</tr>
<tr>
<td></td>
<td>Ground beef (low fat)</td>
<td>4±1</td>
<td>Air</td>
<td>0.437–0.453</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>Ground beef (high fat)</td>
<td>4±1</td>
<td>Air</td>
<td>0.443–0.448</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>Ground beef (low fat)</td>
<td>2</td>
<td>Air</td>
<td>0.57</td>
<td>156</td>
</tr>
<tr>
<td>Streptococcus$^a$</td>
<td>Minced chicken meat</td>
<td>4</td>
<td>Air</td>
<td>0.651</td>
<td>162</td>
</tr>
<tr>
<td>faecalis</td>
<td>Minced chicken meat</td>
<td>4</td>
<td>$\text{CO}_2$</td>
<td>0.702</td>
<td>162</td>
</tr>
<tr>
<td></td>
<td>Minced chicken meat</td>
<td>4</td>
<td>Vacuum</td>
<td>0.697</td>
<td>162</td>
</tr>
<tr>
<td></td>
<td>Minced chicken meat</td>
<td>4</td>
<td>$\text{N}_2$</td>
<td>0.679</td>
<td>162</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>Filet américain</td>
<td>18–20</td>
<td>Air</td>
<td>0.043–0.080</td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>Ground beef</td>
<td>18–20</td>
<td>Air</td>
<td>0.10–0.21</td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>Ground beef</td>
<td>25</td>
<td>Air</td>
<td>0.196</td>
<td>163</td>
</tr>
<tr>
<td></td>
<td>Minced pork</td>
<td>10</td>
<td>Air</td>
<td>0.164–0.204</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>Minced pork</td>
<td>10</td>
<td>$\text{CO}_2$:$\text{N}_2$</td>
<td>0.176–0.187</td>
<td>151</td>
</tr>
</tbody>
</table>

$^a$ Now classified as *Enterococcus*.

mech. deb. = mechanically deboned; NS = not specified.
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°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°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 radiiora* 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 radiiora*, 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

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

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, Angiostongylus 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

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

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

*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

<table>
<thead>
<tr>
<th>Yeast</th>
<th>Initial cell number per ml</th>
<th>Irradiation medium</th>
<th>Irradiation dose required to prevent growth (kGy)</th>
<th>Post-irradiation medium and other conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida crusei</td>
<td>$10^7$</td>
<td>Phosphate buffer</td>
<td>5.5</td>
<td>Malt extract agar, 27°C for up to 15 days</td>
<td>211</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>$10^7$</td>
<td>Phosphate buffer</td>
<td>10</td>
<td>Malt extract agar, 27°C for up to 15 days</td>
<td>211</td>
</tr>
<tr>
<td>Cryptococcus albidos</td>
<td>0.6–2.5 x $10^8$</td>
<td>Grape juice</td>
<td>10</td>
<td>Grape juice, 17°C, for 21 days</td>
<td>212</td>
</tr>
<tr>
<td>Debaryomyces klöckeri</td>
<td>0.6–2.5 x $10^8$</td>
<td>Grape juice</td>
<td>7.5</td>
<td>Grape juice, 17°C for 21 days</td>
<td>212</td>
</tr>
<tr>
<td>Pullularia pullulans</td>
<td>$10^7$</td>
<td>Phosphate buffer</td>
<td>20</td>
<td>Malt extract agar, 27°C for up to 15 days</td>
<td>211</td>
</tr>
<tr>
<td>Rhodotorula glutinis</td>
<td>0.6–2.5 x $10^6$</td>
<td>Grape juice</td>
<td>10</td>
<td>Malt extract agar, 27°C for up to 15 days</td>
<td>212</td>
</tr>
<tr>
<td>Saccharomyces carlsbergiensis</td>
<td>1.5–3.0 x $10^6$</td>
<td>Grape juice</td>
<td>15</td>
<td>Grape juice, 25°C for 19 days</td>
<td>213</td>
</tr>
<tr>
<td>S. cerevisae</td>
<td>1.5–3.0 x $10^6$</td>
<td>Grape juice</td>
<td>18</td>
<td>Grape juice, 25°C for 19 days</td>
<td>213</td>
</tr>
<tr>
<td>S. rosei</td>
<td>1.5–3.0 x $10^6$</td>
<td>Grape juice</td>
<td>15</td>
<td>Grape juice, 25°C for 19 days</td>
<td>213</td>
</tr>
<tr>
<td>Sporobolomyces pararoseus</td>
<td>0.6–2.5 x $10^6$</td>
<td>Nutrient broth</td>
<td>5.0</td>
<td>Nutrient broth, 25°C for 21 days</td>
<td>212</td>
</tr>
<tr>
<td>Torulopsis stellata</td>
<td>1.5–3.0 x $10^6$</td>
<td>Grape juice</td>
<td>10</td>
<td>Grape juice, 25°C for 19 days</td>
<td>213</td>
</tr>
</tbody>
</table>
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 D₁₀-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 D₁₀-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 D₁₀-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 alternatus var. alternatus (formerly A. ochraceus) also reported higher D₁₀-values when gamma-ray irradiation was used.

A D₁₀-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 D₁₀-value of 1.2 kGy in apple juice (222).

D₁₀-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 (10⁶ 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>
<thead>
<tr>
<th>Mould</th>
<th>Initial number of conidia per ml</th>
<th>Irradiation medium</th>
<th>Ionizing radiation</th>
<th>Irradiation dose required to prevent growth (kGy)</th>
<th>Post-irradiation medium and other conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apergillus flavus</td>
<td>$4 \times 10^6$</td>
<td>0.1% peptone + 0.1% Tween 80</td>
<td>Electrons</td>
<td>1.6</td>
<td>Czapek agar, 25°C for 5 days</td>
<td>217</td>
</tr>
<tr>
<td>A. niger</td>
<td>Not given</td>
<td>Malt extract agar</td>
<td>Gamma-rays</td>
<td>2.5</td>
<td>Malt extract agar, 27°C for 10 days</td>
<td>212</td>
</tr>
<tr>
<td>A. parasiticus</td>
<td>$1 \times 10^6$</td>
<td>Water</td>
<td>Gamma-rays</td>
<td>1.6</td>
<td>Autoclaved rice, 26-28°C for 10 days</td>
<td>218</td>
</tr>
<tr>
<td>Alternaria spp.</td>
<td>Not given</td>
<td>Malt extract agar</td>
<td>Gamma-rays</td>
<td>6.0</td>
<td>Malt extract agar, 27°C for 10 days</td>
<td>212</td>
</tr>
<tr>
<td>Botrytis cinerea</td>
<td>Not given</td>
<td>Malt extract agar</td>
<td>Gamma-rays</td>
<td>5.0</td>
<td>Malt extract agar, 27°C for 10 days</td>
<td>212</td>
</tr>
<tr>
<td>Cladosporium spp.</td>
<td>Not given</td>
<td>Malt extract agar</td>
<td>Gamma-rays</td>
<td>6.0</td>
<td>Malt extract agar, 27°C for 10 days</td>
<td>212</td>
</tr>
<tr>
<td>Penicillium viridicatum</td>
<td>$4 \times 10^6$</td>
<td>0.1% peptone + 0.1% Tween 80</td>
<td>Electrons</td>
<td>1.4</td>
<td>Czapek agar, 25°C for 5 days</td>
<td>217</td>
</tr>
</tbody>
</table>
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>
<thead>
<tr>
<th>Mould</th>
<th>Gamma-irradiated (kGy)</th>
<th>Electron-irradiated (kGy)</th>
<th>Values not significantly different (P&lt;0.005, Student t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus echinulatus</em></td>
<td>0.319</td>
<td>0.241</td>
<td></td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>0.276</td>
<td>0.198</td>
<td></td>
</tr>
<tr>
<td><em>A. glaucus</em></td>
<td>0.250</td>
<td>0.243</td>
<td>x</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>0.245</td>
<td>0.199</td>
<td></td>
</tr>
<tr>
<td><em>A. ochraceus</em></td>
<td>0.209</td>
<td>0.198</td>
<td>x</td>
</tr>
<tr>
<td><em>A. versicolor</em></td>
<td>0.282</td>
<td>0.234</td>
<td>x</td>
</tr>
<tr>
<td><em>Penicillium aurantiogriseum</em></td>
<td>0.236</td>
<td>0.194</td>
<td></td>
</tr>
<tr>
<td><em>P. cyclopium</em></td>
<td>0.397</td>
<td>0.290</td>
<td></td>
</tr>
<tr>
<td><em>P. granulactum</em></td>
<td>0.239</td>
<td>0.201</td>
<td></td>
</tr>
<tr>
<td><em>P. roqueforti</em></td>
<td>0.416</td>
<td>0.341</td>
<td></td>
</tr>
<tr>
<td><em>P. verrucosum</em></td>
<td>0.266</td>
<td>0.208</td>
<td></td>
</tr>
<tr>
<td><em>P. viridicatum</em></td>
<td>0.333</td>
<td>0.265</td>
<td>x</td>
</tr>
<tr>
<td><em>Curvularia geniculata</em></td>
<td>1.798</td>
<td>1.193</td>
<td></td>
</tr>
<tr>
<td><em>Alternaria alternata</em></td>
<td>2.409</td>
<td>1.099</td>
<td></td>
</tr>
</tbody>
</table>

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

* 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. steareothermophilus* 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>
<thead>
<tr>
<th>Organism</th>
<th>$D_{10}$ (kGy)</th>
<th>&quot;Shoulder&quot; (kGy)</th>
<th>Irradiation menstruum</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anaerobes:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Clostridium botulinum</em> type A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant strains</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>3.4</td>
<td>3.5</td>
<td>Buffer</td>
<td>230</td>
</tr>
<tr>
<td>62</td>
<td>2.7</td>
<td>3.5</td>
<td>Buffer</td>
<td>142</td>
</tr>
<tr>
<td>Medium-resistant strains</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>2.0</td>
<td>0.7</td>
<td>Buffer</td>
<td>142</td>
</tr>
<tr>
<td>36</td>
<td>1.9</td>
<td>0.7</td>
<td>Buffer</td>
<td>231</td>
</tr>
<tr>
<td>Sensitive strains</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1192y</td>
<td>1.4</td>
<td>0–1.0</td>
<td>Water</td>
<td>232</td>
</tr>
<tr>
<td>NCTC 7272</td>
<td>1.2</td>
<td></td>
<td>Water</td>
<td>233</td>
</tr>
<tr>
<td><em>C. botulinum</em> type B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant strains</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>53</td>
<td>3.3</td>
<td>4.0</td>
<td>Buffer</td>
<td>231</td>
</tr>
<tr>
<td>41</td>
<td>2.1</td>
<td>1.6</td>
<td>Buffer</td>
<td>142</td>
</tr>
<tr>
<td>Medium-resistant strain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1.6</td>
<td>1.8</td>
<td>Buffer</td>
<td>142</td>
</tr>
<tr>
<td>Sensitive strains</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>213</td>
<td>1.1</td>
<td>0.9–1.0</td>
<td>Water</td>
<td>232</td>
</tr>
<tr>
<td>51</td>
<td>1.2</td>
<td>0.0</td>
<td>Buffer</td>
<td>142</td>
</tr>
<tr>
<td><em>C. botulinum</em> type D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.2</td>
<td>2.5–3.5</td>
<td>Buffer</td>
<td>232</td>
<td></td>
</tr>
<tr>
<td><em>C. botulinum</em> type E</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium-resistant strains</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beluga</td>
<td>1.9</td>
<td>1.5</td>
<td>Buffer</td>
<td>142</td>
</tr>
<tr>
<td>Alaska</td>
<td>1.7</td>
<td>2.1</td>
<td>Buffer</td>
<td>142</td>
</tr>
<tr>
<td>16/63</td>
<td>1.6</td>
<td>2.5–3.5</td>
<td>Water</td>
<td>232</td>
</tr>
<tr>
<td>1304</td>
<td>1.7</td>
<td>0.0</td>
<td>Water</td>
<td>234</td>
</tr>
<tr>
<td>Sensitive strains</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beluga</td>
<td>0.8</td>
<td>0.7</td>
<td>Water</td>
<td>232</td>
</tr>
<tr>
<td>8</td>
<td>0.8</td>
<td>0.7</td>
<td>Buffer</td>
<td>136</td>
</tr>
<tr>
<td>V.H.</td>
<td>1.3</td>
<td>1.4</td>
<td>Buffer</td>
<td>142</td>
</tr>
<tr>
<td>Minneapolis</td>
<td>0.8</td>
<td>2.0</td>
<td>Buffer</td>
<td>136</td>
</tr>
<tr>
<td><em>C. botulinum</em> type F</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>2.5–3.5</td>
<td>Water</td>
<td>232</td>
<td></td>
</tr>
<tr>
<td><em>C. perfringens</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>type A</td>
<td>1.2</td>
<td>2.5–3.5</td>
<td>Water</td>
<td>232</td>
</tr>
<tr>
<td>type B</td>
<td>1.7</td>
<td>2.5–3.5</td>
<td>Water</td>
<td>232</td>
</tr>
<tr>
<td>type C</td>
<td>1.8</td>
<td>2.5–3.5</td>
<td>Water</td>
<td>232</td>
</tr>
<tr>
<td>type E</td>
<td>1.2</td>
<td>2.5–3.5</td>
<td>Water</td>
<td>232</td>
</tr>
<tr>
<td><em>C. sporogenes</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA 3679/S2</td>
<td>2.2</td>
<td>2.5–3.5</td>
<td>Water</td>
<td>232</td>
</tr>
<tr>
<td>NCTC 532</td>
<td>1.6</td>
<td>2.5–3.5</td>
<td>Water</td>
<td>232</td>
</tr>
<tr>
<td><strong>Aerobes:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.6</td>
<td>2.0</td>
<td>Water</td>
<td>235</td>
<td></td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.6</td>
<td></td>
<td></td>
<td>Saline (with 5% gelatin)</td>
<td>234</td>
</tr>
<tr>
<td>2.4</td>
<td>0.0</td>
<td>Buffer</td>
<td>236</td>
<td></td>
</tr>
<tr>
<td><em>B. stearothermophilus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>0.0</td>
<td>Buffer</td>
<td>237</td>
<td></td>
</tr>
</tbody>
</table>

* Adapted from Greve et al. (127) and from Goldblith (275) with the permission of the publishers.
Table 14
Radiation resistance of bacterial spores in deep-frozen foods

<table>
<thead>
<tr>
<th>Organism</th>
<th>Food</th>
<th>Irradiation temperature (°C)</th>
<th>D10-value (kGy)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus cereus</em></td>
<td>Mozzarella cheese</td>
<td>-78</td>
<td>3.6</td>
<td>241</td>
</tr>
<tr>
<td></td>
<td>Ice cream</td>
<td>-78</td>
<td>4.1</td>
<td>241</td>
</tr>
<tr>
<td></td>
<td>Yoghurt</td>
<td>-78</td>
<td>4.0</td>
<td>241</td>
</tr>
<tr>
<td><em>Clostridium botulinum</em></td>
<td>type A</td>
<td>Beef</td>
<td>-196</td>
<td>5.9-7.1</td>
</tr>
<tr>
<td></td>
<td>type A + B cocktail</td>
<td>Corned beef</td>
<td>-30±10</td>
<td>1.0-2.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pork sausage</td>
<td>-30±10</td>
<td>0.7-1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Codfish cake</td>
<td>-30±10</td>
<td>0.7-3.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Beef</td>
<td>-30±10</td>
<td>2.5-3.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Roast products</td>
<td>-29</td>
<td>4.0-6.8</td>
</tr>
</tbody>
</table>

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°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*<sub>10</sub>-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<sup>6</sup> spores per strain, to give a total of 10<sup>7</sup> 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 ± 10°C, incubating the cans at +30°C for 6 months, and analysing for swelling, botulinal 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

<table>
<thead>
<tr>
<th>Food</th>
<th>12D value (kGy)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacon</td>
<td>26.5–28.7</td>
<td>243</td>
</tr>
<tr>
<td>Beef</td>
<td>41.2</td>
<td>260</td>
</tr>
<tr>
<td>Chicken</td>
<td>42.7</td>
<td>261</td>
</tr>
<tr>
<td>Corned beef</td>
<td>25.7</td>
<td>242</td>
</tr>
<tr>
<td>Ham</td>
<td>31.4</td>
<td>262</td>
</tr>
<tr>
<td>Pork</td>
<td>43.7</td>
<td>261</td>
</tr>
<tr>
<td>Pork sausage</td>
<td>23.9</td>
<td>242</td>
</tr>
<tr>
<td>Codfish cake</td>
<td>31.7</td>
<td>263</td>
</tr>
</tbody>
</table>

* Irradiated at -30 ± 10 °C, except bacon which was irradiated at 5-25 °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. sporostatic 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 D10-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

<table>
<thead>
<tr>
<th>Suspending medium</th>
<th>Irradiation temperature (°C)</th>
<th>D_{10}-value (kGy)</th>
<th>99% Confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0.5</td>
<td>1.4</td>
<td>1.0–2.1</td>
</tr>
<tr>
<td></td>
<td>-90</td>
<td>5.3</td>
<td>4.7–6.2</td>
</tr>
<tr>
<td>Ground beef</td>
<td>16</td>
<td>7.0</td>
<td>6.6–7.4</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>7.6</td>
<td>7.4–7.9</td>
</tr>
<tr>
<td></td>
<td>-30</td>
<td>6.8</td>
<td>6.3–7.2</td>
</tr>
<tr>
<td></td>
<td>-60</td>
<td>7.8</td>
<td>7.2–8.4</td>
</tr>
<tr>
<td></td>
<td>-90</td>
<td>8.1</td>
<td>7.7–8.5</td>
</tr>
</tbody>
</table>

Reproduced from Sullivan et al. (273) with the permission of the publisher.

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

Massa (274) found D_{10}-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 Brdisher who determined a D_{37}-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 D_{10}-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 D10-value, when irradiated in a rich bacteriological medium, of about 21 kGy; the D10-value for type A toxin was nearer to 40 kGy. Depending on the suspending medium and the assay used, D10-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 D10-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, \textit{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 \textit{in vitro} and \textit{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

<table>
<thead>
<tr>
<th>Source</th>
<th>Packaging</th>
<th>Irradiation</th>
<th>Atm., temp.</th>
<th>Shipping</th>
<th>Storage</th>
<th>Author/reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spent fuel rods:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A, D, S</td>
<td>Canned</td>
<td>Frozen</td>
<td>8 °C</td>
<td></td>
<td></td>
<td>Mead &amp; Griffith (312)</td>
</tr>
<tr>
<td>A, D, S</td>
<td>Canned</td>
<td>Frozen</td>
<td>8 °C</td>
<td></td>
<td></td>
<td>Teply &amp; Kline (313)</td>
</tr>
<tr>
<td>A, D, S</td>
<td>Canned</td>
<td>Frozen</td>
<td>8 °C</td>
<td></td>
<td></td>
<td>Read et al. (314)</td>
</tr>
<tr>
<td>A, D, S</td>
<td>Canned</td>
<td>Frozen</td>
<td>8 °C</td>
<td></td>
<td></td>
<td>Malhotra &amp; Reber (315)</td>
</tr>
<tr>
<td>A, D, S</td>
<td>Canned</td>
<td>Frozen</td>
<td>8 °C</td>
<td></td>
<td></td>
<td>Malhotra &amp; Reber (316)</td>
</tr>
<tr>
<td>A, D, S</td>
<td>Canned</td>
<td>Frozen</td>
<td>8 °C</td>
<td></td>
<td></td>
<td>Malholtra, Reber &amp; Norton (317)</td>
</tr>
<tr>
<td>A, D, S</td>
<td>Canned</td>
<td>Frozen</td>
<td>8 °C</td>
<td></td>
<td></td>
<td>Blood et al. (318)</td>
</tr>
<tr>
<td>A, D, S</td>
<td>Canned</td>
<td>Frozen</td>
<td>rt</td>
<td></td>
<td></td>
<td>Read et al. (319)</td>
</tr>
<tr>
<td>A, D, S</td>
<td>Canned</td>
<td>Frozen</td>
<td>8 °C</td>
<td></td>
<td></td>
<td>Read, Kraybill &amp; Witt (320)</td>
</tr>
<tr>
<td>A, D, S</td>
<td>Dry packed in cans</td>
<td>Frozen</td>
<td>rt</td>
<td></td>
<td></td>
<td>Tinsley, Bone &amp; Bubl (307);</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bone (321)</td>
</tr>
<tr>
<td>A, D, S</td>
<td>Canned chicken and green beans</td>
<td>Frozen</td>
<td>rt</td>
<td></td>
<td></td>
<td>Richardson (322)</td>
</tr>
<tr>
<td>A, D, S</td>
<td>Canned</td>
<td>Frozen</td>
<td>rt</td>
<td></td>
<td></td>
<td>Richardson, Ritchey &amp; Rigdon</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(323); Rigdon (324)</td>
</tr>
<tr>
<td>A, D, S</td>
<td>Canned chicken stew, raw</td>
<td>Frozen</td>
<td>rt/refig.</td>
<td></td>
<td></td>
<td>Phillips, Newcomb &amp; Shanklin</td>
</tr>
<tr>
<td></td>
<td>shredded cabbage, plastic bags</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(325)</td>
</tr>
<tr>
<td></td>
<td>in fibre drum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Canned</td>
<td>Frozen</td>
<td>rt</td>
<td></td>
<td></td>
<td>Radomski et al. (326)</td>
</tr>
<tr>
<td>D</td>
<td>Peaches canned in syrup</td>
<td>Frozen</td>
<td>rt</td>
<td></td>
<td></td>
<td>Tinsley, Bone &amp; Bubl (327)</td>
</tr>
<tr>
<td>A, D, S</td>
<td>Ground pork canned under vacuum</td>
<td>Frozen</td>
<td>rt 3–8 months</td>
<td></td>
<td></td>
<td>Bubl &amp; Butts (328); Bubl (329)</td>
</tr>
<tr>
<td>Source</td>
<td>Packaging</td>
<td>Irradiation</td>
<td>Atm., temp.</td>
<td>Shipping</td>
<td>Storage</td>
<td>Author/reference</td>
</tr>
<tr>
<td>--------</td>
<td>-----------</td>
<td>-------------</td>
<td>-------------</td>
<td>----------</td>
<td>---------</td>
<td>-----------------</td>
</tr>
<tr>
<td>A, D, S</td>
<td>Canned cooked shrimp Oranges</td>
<td>Surface irradiated with electrons at Michigan State University</td>
<td>NS</td>
<td>rt 3–6 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A, D, S</td>
<td>Canned</td>
<td></td>
<td>Frozen</td>
<td>rt</td>
<td>Paynter (332)</td>
<td></td>
</tr>
</tbody>
</table>

**Gamma-rays:**

<table>
<thead>
<tr>
<th>Source</th>
<th>Packaging</th>
<th>Irradiation</th>
<th>Atm., temp.</th>
<th>Shipping</th>
<th>Storage</th>
<th>Author/reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cobalt-60</td>
<td>NS</td>
<td>0.69 kGy/h</td>
<td>NS</td>
<td>NS</td>
<td>Becker et al. (333)</td>
<td></td>
</tr>
<tr>
<td>Cobalt-60</td>
<td>Vacuum packed in cans, frozen PE bags</td>
<td>50 kCl, 0.5 kGy/h</td>
<td>NS</td>
<td>Frozen</td>
<td>rt</td>
<td>McGown, Lewis &amp; Waring (334) Barna (335)</td>
</tr>
<tr>
<td>Cobalt-60</td>
<td>Plastic bags, paper cartons</td>
<td>50 kCl, 0.5 kGy/h</td>
<td>NS</td>
<td>10 °C for up to 2 months</td>
<td>NS</td>
<td>Saint-Lèbe (336)</td>
</tr>
<tr>
<td>Cobalt-60</td>
<td>Plastic bags, paper cartons</td>
<td>50 kCl, 0.5 kGy/h</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>IFIP (337)</td>
</tr>
<tr>
<td>Cobalt-60</td>
<td>NS</td>
<td>2.4 kGy/h</td>
<td>Air, ambient</td>
<td>25–29 °C</td>
<td>Aravindakshan et al. (338) Metwalli (339) van Logten, Berkvens &amp; Krocs (340) van Logten et al. (341)</td>
<td></td>
</tr>
<tr>
<td>Cobalt-60</td>
<td>PE bags</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>Aravindakshan et al. (338) Metwalli (339) van Logten, Berkvens &amp; Krocs (340) van Logten et al. (341)</td>
<td></td>
</tr>
<tr>
<td>Cobalt-60</td>
<td>NS</td>
<td>1.9 kGy/h</td>
<td>NS</td>
<td>NS</td>
<td>Rojo &amp; Fernandez (342)</td>
<td></td>
</tr>
<tr>
<td>Source</td>
<td>Packaging</td>
<td>Irradiation</td>
<td>Atm., temp.</td>
<td>Shipping</td>
<td>Storage</td>
<td>Author/reference</td>
</tr>
<tr>
<td>------------</td>
<td>----------------------------</td>
<td>-----------------</td>
<td>-------------</td>
<td>----------</td>
<td>---------</td>
<td>---------------------------------------</td>
</tr>
<tr>
<td>Machine:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>McGown, Lewis &amp; Waring (334)</td>
</tr>
<tr>
<td>Electron</td>
<td>Vacuum-packed in pouches,</td>
<td>10 MeV,</td>
<td>Frozen</td>
<td>rt</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>frozen</td>
<td>5 µs pulse,</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>180 pulse/s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/4-inch slabs, frozen</td>
<td>NS</td>
<td>Frozen</td>
<td>rt</td>
<td>8°C</td>
<td>Teply &amp; Kline (313)</td>
</tr>
<tr>
<td></td>
<td>Oil in bottle</td>
<td>NS</td>
<td>refriger.</td>
<td></td>
<td></td>
<td>Teply &amp; Kline (313)</td>
</tr>
<tr>
<td></td>
<td>PE bags</td>
<td>10 MeV,</td>
<td>NS</td>
<td></td>
<td>NS</td>
<td>Renner &amp; Reichelt (343)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>1 MeV</td>
<td>25°C</td>
<td></td>
<td>NS</td>
<td>Lang (344)</td>
</tr>
<tr>
<td></td>
<td>(van der Graaf)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fresh in PE bags</td>
<td>3 MeV</td>
<td>NS</td>
<td>Frozen</td>
<td></td>
<td>Verschuuren, van Esch &amp; van Kooy (345)</td>
</tr>
</tbody>
</table>

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; refriger. = refrigerated; rt = room temperature; S = Aiken, SC, United States of America; temp. = temperature.
<table>
<thead>
<tr>
<th>Source</th>
<th>Packaging</th>
<th>Irradiation</th>
<th>Atm., temp.</th>
<th>Shipping</th>
<th>Storage</th>
<th>Author/reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spent fuel rods:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A, D, S</td>
<td>Canned</td>
<td>Frozen</td>
<td>5°C first</td>
<td></td>
<td>rt 3-9 months</td>
<td>McKee et al. (346);</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6 months,</td>
<td>rt to end</td>
<td></td>
<td>Dixon et al. (347)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A, D, S</td>
<td>Canned</td>
<td>NS</td>
<td>rt 3-9 months</td>
<td></td>
<td></td>
<td>Deichmann (348);</td>
</tr>
<tr>
<td></td>
<td>Canned</td>
<td>Frozen</td>
<td></td>
<td></td>
<td></td>
<td>Radomski et al. (349)</td>
</tr>
<tr>
<td>A, D, S</td>
<td>Canned</td>
<td>Frozen</td>
<td>rt</td>
<td></td>
<td></td>
<td>Calandra &amp; Kay (350)</td>
</tr>
<tr>
<td>A, D, S</td>
<td>Canned</td>
<td>Frozen</td>
<td>8°C</td>
<td></td>
<td></td>
<td>Teply &amp; Kline (313)</td>
</tr>
<tr>
<td>A, D, S</td>
<td>Canned</td>
<td>Frozen</td>
<td>rt</td>
<td></td>
<td></td>
<td>Monsen (351-353)</td>
</tr>
<tr>
<td>A, D, S</td>
<td>Canned</td>
<td>Frozen</td>
<td>rt</td>
<td></td>
<td></td>
<td>Thompson et al. (354, 355)</td>
</tr>
</tbody>
</table>

Gamma-rays:

<table>
<thead>
<tr>
<th>Source</th>
<th>Packaging</th>
<th>Irradiation</th>
<th>Atm., temp.</th>
<th>Shipping</th>
<th>Storage</th>
<th>Author/reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cobalt-60</td>
<td>Heated, canned</td>
<td>NS</td>
<td>Frozen</td>
<td></td>
<td>rt</td>
<td>Raltech Scientific Services (356, 357)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Maffei, Mazzali &amp; DeSantis (358)</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td>NS</td>
<td>Biagini et al. (359)</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>6 kGy/h</td>
<td>NS</td>
<td></td>
<td>NS</td>
<td>Saint-Lèbe (336)</td>
</tr>
<tr>
<td></td>
<td>Plastic bags,</td>
<td>50 kCi,</td>
<td>NS</td>
<td></td>
<td>NS</td>
<td>Porter &amp; Festing (360)</td>
</tr>
<tr>
<td></td>
<td>paper cartons</td>
<td>0.5 kGy/h</td>
<td></td>
<td></td>
<td>NS</td>
<td>Bugyaki et al. (361)</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>1.8 kGy/h,</td>
<td></td>
<td></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ambient</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caesium-137</td>
<td>Plastic bags, paper</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td>NS</td>
<td>Saint-Lèbe (336)</td>
</tr>
<tr>
<td></td>
<td>cartons</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source</td>
<td>Packaging</td>
<td>Irradiation</td>
<td>Atm., temp.</td>
<td>Shipping</td>
<td>Storage</td>
<td>Author/reference</td>
</tr>
<tr>
<td>-----------------</td>
<td>----------------------------</td>
<td>-------------</td>
<td>-------------</td>
<td>----------</td>
<td>---------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Machine:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electron</td>
<td>Heated, vacuum-packed</td>
<td>10 MeV</td>
<td>Frozen</td>
<td>rt</td>
<td></td>
<td>Raltech Scientific Services (356, 357)</td>
</tr>
<tr>
<td></td>
<td>in pouches</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/4-inch slabs, frozen</td>
<td>NS</td>
<td>Frozen</td>
<td></td>
<td>8 °C</td>
<td>Teply &amp; Kline (313)</td>
</tr>
<tr>
<td></td>
<td>Oil in bottle</td>
<td>NS</td>
<td>refrig.</td>
<td>rt</td>
<td></td>
<td>Teply &amp; Kline (313)</td>
</tr>
</tbody>
</table>

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**

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

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

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>
<thead>
<tr>
<th>Source</th>
<th>Packaging</th>
<th>Irradiation</th>
<th>Atm., temp.</th>
<th>Storage</th>
<th>Author/reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gamma-rays:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cobalt-60</td>
<td>Plastic film bags</td>
<td>17 kGy/h</td>
<td>4°C, ambient</td>
<td>1, 2, 7, 14, 21 days</td>
<td>Joner, Underdal &amp; Lunde (382)</td>
</tr>
<tr>
<td>Sealed ampoules</td>
<td>NS</td>
<td>6.5 kCl</td>
<td>NS</td>
<td>NS</td>
<td>Neimand et al. (383)</td>
</tr>
<tr>
<td>Powder form in individual containers, packed in plastic 13-cm diam. containers</td>
<td>NS</td>
<td>4.5 kGy/h</td>
<td>Ambient</td>
<td></td>
<td>Bugyaki, Lafontaine &amp; Moutschen-Dahmen (384)</td>
</tr>
<tr>
<td>Plastic film</td>
<td>NS</td>
<td>4°C, ambient</td>
<td>1, 7, 14 days</td>
<td></td>
<td>Vijayalaxmi (385)</td>
</tr>
<tr>
<td>Unsealed plastic pouches in metal cans</td>
<td>NS</td>
<td>NS</td>
<td>Aerobic, ambient</td>
<td>Ambient</td>
<td>Joner &amp; Underdal (386)</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>Aerobic, ambient</td>
<td>NS</td>
<td>Münzer &amp; Renner (387)</td>
</tr>
<tr>
<td>400-ml beaker</td>
<td>NS</td>
<td>3.6 kGy/h</td>
<td>25°C</td>
<td>NS</td>
<td>Central Food Research Institute (388)</td>
</tr>
<tr>
<td>Solution</td>
<td>NS</td>
<td>3.3 kGy/h</td>
<td>NS</td>
<td>NS</td>
<td>Farkas, Andrássy &amp; Incze (389)</td>
</tr>
<tr>
<td>N₂ flush, frozen, seal; dry ice</td>
<td>NS</td>
<td>4.5 kGy/h</td>
<td>Air</td>
<td>6-8°C up to 25 weeks</td>
<td>Schubert et al. (390)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 min</td>
<td></td>
<td></td>
<td>Shaw &amp; Hayes (391)</td>
</tr>
</tbody>
</table>

| Machine: |
| Electron | NS | 10 MeV | NS | NS | Münzer (394) |

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

* 2% solution concentrated to 20% for shipment, diluted with saline before use.
<table>
<thead>
<tr>
<th>Source</th>
<th>Packaging</th>
<th>Irradiation</th>
<th>Atm., temp.</th>
<th>Storage</th>
<th>Author/reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gamma-rays:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cobalt-60</td>
<td>Canned, frozen</td>
<td>3 MCi</td>
<td>Frozen</td>
<td>rt</td>
<td>Mittler (395)</td>
</tr>
<tr>
<td>Cobalt-60</td>
<td>Canned</td>
<td>NS</td>
<td>Frozen</td>
<td>NS</td>
<td>Lusskin (396)</td>
</tr>
<tr>
<td>Cobalt-60</td>
<td>Canned</td>
<td>NS</td>
<td>Frozen</td>
<td>rt</td>
<td>Raltech Scientific Services (397)</td>
</tr>
<tr>
<td>Cobalt-60</td>
<td>PE bags</td>
<td>57 Gy/min</td>
<td>Air, ambient</td>
<td>25°C</td>
<td>Varma et al. (398-401)</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>Moutschen-Dahmen, Moutschen &amp; Ehrenberg (402)</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
<td>8.5 kGy/h</td>
<td>NS</td>
<td>NS</td>
<td>Johnston-Arthur et al. (403)</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
<td>2.4 kGy/h</td>
<td>4-6°C</td>
<td>3-4 weeks</td>
<td>Chauhan et al. (404)</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
<td>2.4 kGy/h</td>
<td>NS</td>
<td>NS</td>
<td>Chauhan et al. (405)</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
<td>11-11.5h</td>
<td>Ambient</td>
<td>37-38°C</td>
<td>Leonard, Wilcox &amp; Schietecatte (406)</td>
</tr>
<tr>
<td>NS</td>
<td>250 g pellets in PE sacks</td>
<td>NS</td>
<td>Ambient</td>
<td>22-24°C</td>
<td>Johnson-Arthur et al. (407)</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>Anderson et al. (408)</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
<td>14 kCl, 70 min</td>
<td>NS</td>
<td>NS</td>
<td>Chopra (409)</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>Aerobic, ambient</td>
<td>NS</td>
<td>Central Food Research Institute (388)</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
<td>3.1 kGy/h</td>
<td>NS</td>
<td>Fed 8-18 days after irradiation</td>
<td>Chaubey et al. (410)</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
<td>Aerobic, ambient</td>
<td>NS</td>
<td>NS</td>
<td>Farkas &amp; Andrássy (411)</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
<td>Aerobic, ambient</td>
<td>NS</td>
<td>Fed 5-9 days after irradiation</td>
<td>Farkas, Andrássy &amp; Incze (389)</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
<td>0.5 kGy/h</td>
<td>NS</td>
<td>Fed 8-18 days after irradiation</td>
<td>Barna (412)</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
<td>3.6 kGy/h</td>
<td>Ambient</td>
<td>25°C</td>
<td>Schubert et al. (390)</td>
</tr>
<tr>
<td>Source</td>
<td>Packaging</td>
<td>Irradiation</td>
<td>Atm., temp.</td>
<td>Storage</td>
<td>Author/reference</td>
</tr>
<tr>
<td>----------</td>
<td>--------------------</td>
<td>-------------</td>
<td>----------------------</td>
<td>---------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>NS</td>
<td>4.5 kGy/h</td>
<td>N₂, 10 min, seal</td>
<td>6-8°C for up to 25 weeks</td>
<td>NS</td>
<td>Aiyar &amp; Rao (393)</td>
</tr>
<tr>
<td>Plastic bags</td>
<td>180 krad/h</td>
<td>N₂ flush</td>
<td>NS</td>
<td>NS</td>
<td>Bugyaki et al. (361)</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
<td>Ambient, N₂ flush</td>
<td>NS</td>
<td>NS</td>
<td>Tanaka et al. (413)</td>
</tr>
<tr>
<td>Caesium-137</td>
<td>NS</td>
<td>4 kGy/h</td>
<td>rt</td>
<td>rt</td>
<td>Bernades et al. (414)</td>
</tr>
</tbody>
</table>

Machine:

<table>
<thead>
<tr>
<th>Electron</th>
<th>Vacuum-packed in pouches</th>
<th>10 MeV</th>
<th>Frozen</th>
<th>rt</th>
<th>Mittler (395)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vacuum-packed in pouches</td>
<td>NS</td>
<td>Frozen</td>
<td>rt</td>
<td>Lusskin (396)</td>
<td></td>
</tr>
<tr>
<td>Vacuum-packed in pouches</td>
<td>NS</td>
<td>Frozen</td>
<td>rt</td>
<td>Raltech Scientific Services (397)</td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>10 MeV, 10 min</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>Eriksen &amp; Emborg (415)</td>
</tr>
<tr>
<td>Open aluminium trays</td>
<td>10 MeV</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>Münzer &amp; Renner (416)</td>
</tr>
<tr>
<td>NS</td>
<td>10 MeV</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>Renner (417)</td>
</tr>
<tr>
<td>NS</td>
<td>10 MeV</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>Münzer &amp; Renner (418)</td>
</tr>
<tr>
<td>Source</td>
<td>Packaging</td>
<td>Irradiation</td>
<td>Atm., temp.</td>
<td>Storage</td>
<td>Author/reference</td>
</tr>
<tr>
<td>------------------------</td>
<td>------------------------------------------------</td>
<td>-------------------------------------------------</td>
<td>-------------</td>
<td>-----------------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>NS</td>
<td></td>
<td>10 MeV, 10 min</td>
<td>NS</td>
<td>NS</td>
<td>Renner et al. (421)</td>
</tr>
<tr>
<td>Unsealed plastic</td>
<td></td>
<td>10 MeV, 10 min</td>
<td>Ambient</td>
<td>NS</td>
<td>Münzer &amp; Renner (387)</td>
</tr>
<tr>
<td>pouches in metal</td>
<td>in metal cans</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-ray</td>
<td>Medium in lucite vials</td>
<td>25 MeV electrons (600 mA) on tungsten converter, 10 kGy/min</td>
<td>NS</td>
<td>Used immediately or 3 weeks</td>
<td>Rinehart &amp; Ratty (419)</td>
</tr>
<tr>
<td>Medium in</td>
<td>lucite vials</td>
<td>250 kV$_p$, 15 mA, 50 Gy/min</td>
<td>NS</td>
<td>Used immediately or 3 weeks</td>
<td>Rinehart &amp; Ratty (419)</td>
</tr>
<tr>
<td>sucrose solution in</td>
<td>lucite vials</td>
<td></td>
<td></td>
<td>Used immediately or 3 weeks, diluted to 10% with medium</td>
<td>Rinehart &amp; Ratty (420)</td>
</tr>
<tr>
<td>Herring sperm DNA</td>
<td>lucite vials</td>
<td>250 kV$_p$, 15 mA, 50 Gy/min</td>
<td>NS</td>
<td>Used immediately or 3 weeks, diluted to 10% with medium</td>
<td>Rinehart &amp; Ratty (420)</td>
</tr>
</tbody>
</table>

Atm. = atmosphere; NS = not specified; PE = polyethylene; rt = room temperature; temp. = temperature.
<table>
<thead>
<tr>
<th>Study type</th>
<th>Duration</th>
<th>Author/reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subchronic</td>
<td>90 days</td>
<td>Malhotra &amp; Reber (315)</td>
</tr>
<tr>
<td></td>
<td>8 and 9 weeks</td>
<td>Malhotra &amp; Reber (316)</td>
</tr>
<tr>
<td></td>
<td>8 and 14 weeks</td>
<td>Malhotra, Reber &amp; Norton (317)</td>
</tr>
<tr>
<td></td>
<td>8–12 weeks</td>
<td>Read et al. (319)</td>
</tr>
<tr>
<td></td>
<td>8–12 weeks</td>
<td>Read, Kraybill &amp; Witt (320)</td>
</tr>
<tr>
<td></td>
<td>54 days</td>
<td>McGown, Lewis &amp; Waring (334)</td>
</tr>
<tr>
<td></td>
<td>200 days</td>
<td>Rojo &amp; Fernandez (342)</td>
</tr>
<tr>
<td></td>
<td>84 days</td>
<td>Brin, Ostashever &amp; Kalinsky (330)</td>
</tr>
<tr>
<td></td>
<td>280 days</td>
<td>Lang (344)</td>
</tr>
<tr>
<td></td>
<td>84 days</td>
<td>Verschuuren, van Esch &amp; van Kaay (345)</td>
</tr>
<tr>
<td></td>
<td>90 days</td>
<td>van Logten et al. (340)</td>
</tr>
<tr>
<td></td>
<td>120 days</td>
<td>Metwalli (339)</td>
</tr>
<tr>
<td>Reproduction</td>
<td>Teratology</td>
<td>IFIP (338)</td>
</tr>
<tr>
<td>Reproduction</td>
<td>15 days</td>
<td></td>
</tr>
<tr>
<td>Carcinogenesis</td>
<td>2 years</td>
<td>Teply &amp; Kline (313)</td>
</tr>
<tr>
<td></td>
<td>2 years</td>
<td>Bone (321)</td>
</tr>
<tr>
<td></td>
<td>2 years</td>
<td>Teply &amp; Kline (313)</td>
</tr>
<tr>
<td></td>
<td>2.5 years</td>
<td>van Logten et al. (341)</td>
</tr>
<tr>
<td>Combined carcinogenesis and reproduction</td>
<td>2 years</td>
<td>Mead &amp; Griffith (312)</td>
</tr>
<tr>
<td></td>
<td>2 years</td>
<td>Teply &amp; Kline (313)</td>
</tr>
<tr>
<td></td>
<td>2 years</td>
<td>Read et al. (314)</td>
</tr>
<tr>
<td></td>
<td>2 years</td>
<td>Blood et al. (318)</td>
</tr>
<tr>
<td></td>
<td>2 years</td>
<td>Becker et al. (333)</td>
</tr>
<tr>
<td></td>
<td>2 years</td>
<td>Richardson (323)</td>
</tr>
<tr>
<td></td>
<td>2 years</td>
<td>Richardson, Ritchey &amp; Rigdon (323); Rigdon (324)</td>
</tr>
<tr>
<td></td>
<td>2 years</td>
<td>Phillips, Newcomb &amp; Shanklin (325)</td>
</tr>
<tr>
<td></td>
<td>2 years</td>
<td>Tinsley, Bone &amp; Bubl (307); Bone (321)</td>
</tr>
<tr>
<td></td>
<td>2 years</td>
<td>Barna (335)</td>
</tr>
<tr>
<td></td>
<td>3 generations</td>
<td>Saint-Lèbe (336)</td>
</tr>
<tr>
<td></td>
<td>2 years</td>
<td>Radomski et al. (326)</td>
</tr>
<tr>
<td></td>
<td>3 years</td>
<td>Renner &amp; Reichelt (343)</td>
</tr>
<tr>
<td></td>
<td>2 years</td>
<td>Tinsley, Bone &amp; Bubl (327)</td>
</tr>
<tr>
<td></td>
<td>2 years</td>
<td>Bubl &amp; Butts (328); Bubl (329)</td>
</tr>
<tr>
<td></td>
<td>2 years</td>
<td>Phillips, Newcomb &amp; Shanklin (331)</td>
</tr>
<tr>
<td></td>
<td>2 years</td>
<td>Aravindakshan et al. (338)</td>
</tr>
<tr>
<td></td>
<td>2 years</td>
<td>Paynter (332)</td>
</tr>
</tbody>
</table>
### Table 24
**High-dose irradiation study types – mouse studies**

<table>
<thead>
<tr>
<th>Study type</th>
<th>Duration</th>
<th>Author/reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subchronic</td>
<td>60 days</td>
<td>Maffei, Mazzali &amp; DeSantis (358)</td>
</tr>
<tr>
<td>Chronic</td>
<td>14 months, 19 months</td>
<td>Teply &amp; Kiine (313), Monsen (351-353)</td>
</tr>
<tr>
<td>Reproduction</td>
<td>Repro. and teratology 20 days, Lifetime, 3 generations, Repro. and teratology 200 days</td>
<td>Raltech Scientific Services (356), Saint-Lèbe (336), Porter &amp; Festing (360)</td>
</tr>
<tr>
<td>Carcinogenesis</td>
<td>750 days, 12-28 months, 2 years, 730 days</td>
<td>McKee et al. (346); Dixon et al. (347), Deichmann (348); Radomski et al. (349), Calandra &amp; Kay (350), Raltech Scientific Services (357)</td>
</tr>
<tr>
<td>Combined carcinogenesis and reproduction</td>
<td>730 days, 300 and 600 days, lifetime, Lifetime, 3 generations</td>
<td>Biagini et al. (359), Thompson et al. (354, 355), Buggyaki et al. (361)</td>
</tr>
</tbody>
</table>
### Table 25
**High-dose irradiation study types – dog studies**

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

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

<table>
<thead>
<tr>
<th>Study type</th>
<th>Test species</th>
<th>Duration</th>
<th>Author/reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subchronic</td>
<td>Japanese quail</td>
<td>26 days</td>
<td>Koch et al. (377); Döllstädt et al. (378)</td>
</tr>
<tr>
<td></td>
<td>Pigs</td>
<td>16 weeks, 90 days</td>
<td>Strik (380)</td>
</tr>
<tr>
<td></td>
<td>Chicks</td>
<td>5 weeks</td>
<td>Takigawa, Danbara &amp; Ohyama (381)</td>
</tr>
<tr>
<td>Chronic</td>
<td>Rhesus monkeys</td>
<td>24 months</td>
<td>Blood et al. (376)</td>
</tr>
<tr>
<td>Reproduction</td>
<td>Hamsters, reproduction</td>
<td>5 days</td>
<td>Raltech Scientific Services (379)</td>
</tr>
<tr>
<td></td>
<td>and teratology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined</td>
<td>Pigs</td>
<td>3 generation reproduction</td>
<td>Strik (380)</td>
</tr>
<tr>
<td>carcino genesis and</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>reproduction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food type (%) in diet</td>
<td>Study type/ duration</td>
<td>Irradiation dose (kGy)</td>
<td>Process conditions</td>
</tr>
<tr>
<td>----------------------</td>
<td>----------------------</td>
<td>------------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Bacon (b) (35%)</td>
<td>2 years P, F₁, F₂, generations</td>
<td>C = 0 H = 27.9 VH = 55.8</td>
<td>Spent fuel rods; foods canned, shipped frozen; irradiated at 60°F; stored at rt</td>
</tr>
<tr>
<td>Beef (35%)</td>
<td>90 days</td>
<td>C = 0 VH = 55.8</td>
<td>Spent fuel rods; foods canned, shipped frozen; irradiated at 60°F; stored at rt</td>
</tr>
<tr>
<td>Beef (35%)</td>
<td>9 weeks (3 exp.), 8 weeks (1 exp)</td>
<td>C = 0 VH = 55.8</td>
<td>Spent fuel rods; foods canned, shipped frozen; irradiated at 60°F; stored at rt</td>
</tr>
<tr>
<td>Beef (35%)</td>
<td>14 weeks (exp I), 8 weeks (exp II)</td>
<td>C = 0 VH = 55.8</td>
<td>Spent fuel rods; foods canned, shipped frozen; irradiated at 60°F; stored at rt</td>
</tr>
<tr>
<td>Food type (% in diet)</td>
<td>Study type/duration</td>
<td>Irradiation dose (kGy)</td>
<td>Process conditions</td>
</tr>
<tr>
<td>----------------------</td>
<td>---------------------</td>
<td>------------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>Beef (35%)</td>
<td>2 years P, F₁⁻F₃ generations</td>
<td>C = 0 H = 27.9 VH = 55.8</td>
<td>Spent fuel rods; foods canned, shipped frozen; irradiated at 60 °F; stored at rt</td>
</tr>
<tr>
<td>Beef (35%); raw frozen (rf), raw stored (rs) at 70 or 100 °F, cooked stored (cs) at 70 °F.</td>
<td>8–12 weeks C = 0 H = 27.9 VH = 55.8</td>
<td></td>
<td>Spent fuel rods; canned meats, shipped frozen; irradiated at 60 °F; stored at rt</td>
</tr>
<tr>
<td>Beef stew (35%)</td>
<td>2 years P, F₁⁻F₃ generations</td>
<td>C = 0 H = 27.9 VH = 55.8</td>
<td>Spent fuel rods (Dugway)</td>
</tr>
<tr>
<td>Beef, fresh ham, haddock, turkey, bacon, corn, spinach, beets, snap beans, peaches, strawberry, bread, cereal bar and powdered milk (14 food items, 35% each)</td>
<td>8–12 weeks C = 0 H = 30 VH = 60</td>
<td></td>
<td>Spent fuel rods; foods canned, shipped frozen; irradiated at 60 °F; stored at rt</td>
</tr>
<tr>
<td>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</td>
<td>2 years P, F₁⁻F₂ generations</td>
<td>C = 0 H = 16.8 60Co 0.69 kGy/h; storage NS</td>
<td></td>
</tr>
<tr>
<td>Food type (%) in diet&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Study type/duration</td>
<td>Irradiation dose (kGy)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Process conditions</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>---------------------</td>
<td>----------------------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Cabbage (ca) (35%) stored in ice-packed fibre drums, at 34°F, 70% RH</td>
<td>2 years P, F&lt;sub&gt;1&lt;/sub&gt;–F&lt;sub&gt;3&lt;/sub&gt; generations</td>
<td>C = 0 H = 27.9 VH = 55.8 (ca 2.8 and 5.6)</td>
<td>Spent fuel rods; raw, shredded (ca), plastic bags in fibre drums (Dugway)</td>
</tr>
<tr>
<td>Carrot (35%): control stored at 0°C; irradiated turned dark, sometimes surrounded by jelly-like substance, smelled acid</td>
<td>2 years P, F&lt;sub&gt;1&lt;/sub&gt;–F&lt;sub&gt;2&lt;/sub&gt; generations</td>
<td>C = 0 H = 27.9 VH = 55.8</td>
<td>Spent fuel rods; carrots sliced, dried-packed in cans; stored at rt</td>
</tr>
<tr>
<td>Chicken (35%): Frozen (f), Thermal (t), Gamma (g), Electron (e)</td>
<td>54 days</td>
<td>C = 0 VH = 59</td>
<td>&lt;sup&gt;60&lt;/sup&gt;Co: vacuum-packed in cans, frozen; 10 MeV electrons; 5-µs pulse, 180 pulse/s; vacuum-packed in pouches, frozen</td>
</tr>
<tr>
<td>Chicken (ch) (35%), synthetic diet</td>
<td>2 years P, F&lt;sub&gt;1&lt;/sub&gt;–F&lt;sub&gt;3&lt;/sub&gt; generations</td>
<td>C = 0 H = 27.9 VH = 55.8</td>
<td>Spent fuel rods; canned and shipped frozen; irradiated at 60°F; stored at rt</td>
</tr>
<tr>
<td>Food type (food type</td>
<td>Study duration</td>
<td>Irradiation dose (kGy)</td>
<td>Process conditions</td>
</tr>
<tr>
<td>-------------------</td>
<td>----------------</td>
<td>------------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Chicken (ch) (35%) and green beans (gb) (35%): synthetic diet</td>
<td>2 years P, F₁-F₃ generations</td>
<td>C = 0 H = 27.9 VH = 55.8</td>
<td>Spent fuel rods; canned (ch) and (gb) shipped frozen; irradiated at 60°F; stored at rt</td>
</tr>
<tr>
<td>Chicken stew (cs) (35%); control cs received frozen, stored frozen</td>
<td>2 years P, F₁-F₃ generations</td>
<td>C = 0 H = 27.9 VH = 55.8 cabbage (ca 2.8 and 5.6)</td>
<td>Spent fuel rods; canned (Dugway)</td>
</tr>
<tr>
<td>Corn (co) (35%); control co received frozen, stored frozen</td>
<td>2 years P, F₁-F₃ generations</td>
<td>C = 0 H = 27.9 VH = 55.8</td>
<td>Spent fuel rods; canned, irradiated at 60°F; stored at rt</td>
</tr>
<tr>
<td>Evaporated milk (35%)</td>
<td>2 years P, F₁-F₃ generations</td>
<td>C = 0 H = 27.9 VH = 55.8</td>
<td>Spent fuel rods; (Dugway)</td>
</tr>
<tr>
<td>Fruit compote (!) (35%)</td>
<td>2 years P, F₁-F₃ generations</td>
<td>C = 0 H = 27.9 VH = 55.8</td>
<td>Spent fuel rods; foods canned, shipped frozen; irradiated at 60°F; stored at rt</td>
</tr>
</tbody>
</table>