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food - science and techniques

Reports of the Scientific Committee for Food

(Eighteenth series)

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TABLE OF CONTENTS

I. TERMS OF REFERENCE AND CONCLUSIONS

TERMS OF REFERENCE

To advise on the wholesomeness of foods irradiated by suitable procedures.

BACKGROUND[a]

The initial research into the scientific and technological aspects of food preservation and sterilization by irradiation as a credible alternative technology was carried out in the USA in the late '40s. At the same time, concern arose over the wholesomeness of food preserved in this manner and many individual irradiated foods were investigated as if they were food additives. It was not surprising therefore, to find that a large number of expensive, lengthy and sometimes repetitive animal studies were being carried out in a number of countries.

To rationalize and coordinate these various efforts in a more productive fashion, the International Project in the Field of Food Irradiation was set up in 1971 as a result of an agreement between 19 interested countries under the joint sponsorship of the International Atomic Energy Agency (IAEA) and the Food and Agricultural Organization (FAO), both UNO agencies, and the European Nuclear Energy Agency, later renamed the OECD Nuclear Energy Agency. The Federal Republic of Germany provided Host Centre facilities at Karlsruhe. Membership soon rose to 24 countries by 1975 and remained at that level until the termination of the Project on 31 December 1981.

The objectives of the Project were essentially the carrying out of a research programme into methodology at the Host Centre and the coordination, including supervision, of wholesomeness testing and related studies in laboratory animals, contracted out to reputable contract laboratories on behalf of the membership of the Project. The Project placed some 12 extensive feeding studies with contract laboratories to investigate various toxicological aspects of some irradiated foods in order to fulfil the requests of the 1969 and 1976 Joint FAO/IAEA/WHO Expert Committees on Irradiated Food which have assessed the clearance of the irradiation process and of the irradiated foods from the point of view of safety to health. After 1976 a sensitive methodology was developed in the Project's own laboratory based on simple short-term mutagenicity tests on digests of irradiated foods. These biological investigations supplemented extensive coordinated programmes of research into the radiation chemistry of food and food components carried out in some 9 collaborating specialist laboratories in the world. Data were collected on the identification and quantitative measurements of radiolytic products derived from the major components of irradiated foods and compared with the effects of conventional food processing.

As a result of all these efforts, the 1980 Joint FAO/IAEA/WHO Expert Committee accepted the safety of the process of irradiation for the preservation of food up to an overall average dose of 10 kGy[+].

As a consequence of this decision, the Codex Alimentarius Commission developed a General Standard for Irradiated Food and Code of Practice for the Operation of Radiation Facilities for irradiated foods in international trade. Thus, having achieved its objectives, the International Project was wound up.

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[a] see also Section II

[+] 1 kGy = 100 krad = 0.1 Mrad = 1000 Joule absorbed per kg of mass

During its existence the Project issued 12 volumes of a bulletin entitled "Food Irradiation Information" and 67 Technical Reports on the various wholesomeness studies carried out under contract or performed in the laboratory at the Host Centre. A number of scientific papers were published in the open scientific literature. Two extensive monographs entitled "Radiation Chemistry of Major Food Components" and "Recent Advances in Food Irradiation" were published in book form in 1977 and 1983 respectively. Moreover, the International Project accumulated an extensive documentation on the wholesomeness aspects and the radiation chemistry of foods and food ingredients. It also provided a survey of all literature relating to the wholesomeness aspects of irradiated foods published since 1950 in a computerized form at the Host Centre in Karlsruhe. In addition, since 1955 the Host Centre has issued a bibliography on the preservation of foodstuffs by ionizing radiation covering the compilation and evaluation of all relevant literature.

Because of the availability of original data and publications, two meetings of the EEC Scientific Committee for Food were held in Karlsruhe during the preparation of this Report.

The Committee included within its terms of reference not only the evaluation of potential health aspects directly related to toxicological and nutritional properties of irradiated foods but also the possible pathogenic and food-spoilage properties of organisms surviving radiation processing of food. The Committee did not review in detail the extensive data on processing of foods (e.g. beef and poultry) with doses higher than 10 kGy for sterilization purposes because the radiation conditions under which they were obtained are not relevant to the likely commercial applications of food irradiation. Effectively, therefore, this report concentrates on radiation processing at doses of the order of 10 kGy or less. The Committee has not considered the specific issues relating to the irradiation of food additives and food packaging materials.

RADIATION DOSES AND EFFECTS[b]

Processing with doses of radiation between 0.02 and 1 kGy can influence a variety of biological processes. For instance, it may induce inhibition of sprouting during storage (e.g. of onions and potatoes) and delay of ripening (e.g. of mangoes and papayas). As radiation doses in this range kill the insects at all stages of their life cycle, they can also be used to control insect infestation (e.g. of wheat, rice, pulses and dates) thus providing an alternative to pesticides or fumigants.

Processing of foods (e.g. fish products, chicken, strawberry, spices and condiments) with doses between 1 and 10 kGy may be used for practical elimination of pathogenic organisms (radicidation) and of non-spore-forming microorganisms other that viruses (radurization). It appears that irradiation in this higher dose range also provides an alternative to some chemical treatments (e.g. ethylene oxide) to reduce microbial contamination in spices, dried vegetables and thickeners.

It is worth noting that not all food items are suitable for radiation processing; for instance, irradiation of milk and milk-derived products may facilitate the development of rancidity through induction of lipid peroxidation.

MICROBIOLOGICAL ASPECTS[c]

The major benefits of radicidation and radurization are associated with the possibility of controlling many rather common health hazards related to several food-borne parasitic

[b] See also Section III.1.

[c] See also Section VII.

diseases such as trichinosis, taeniasis, and those associated with the presence of Salmonella, Campylobacter and Toxoplasma in meat and poultry or of Shigella, Vibrio parahaemolyticus and enteropathogenic Escherichia coli in deep frozen sea food.

Because of the variations in radiation resistance of microorganisms, irradiation at doses up to 10 kGy, in spite of its usefulness, cannot in all foods solve by itself all the problems related to the microbiological safety and keeping quality of foods. Solution of such problems may in some cases require appropriate combination treatment, e.g. irradiation plus heat treatment, irradiation plus chemical preservation, or appropriate storage conditions after irradiation including proper storage temperature and packaging. It should be emphasized that irradiation, besides itself creating barriers to the transmission of pathogenic organisms through food, especially Gram-negative organisms, also renders the survivors of irradiation usually more sensitive to heat, drying and other technological treatments of food.

The problems due to suppression of spoilage organisms by means of radiation processing at low doses are likely to be no greater than those encountered with other methods of preservation, e.g. pasteurization, curing and vacuum packing. Moreover, no public health problems can be attributed to aspects such as possible enhanced pathogenicity, enhanced radiation resistance and changes of taxonomically-relevant characteristics of microorganisms surviving after food irradiation at low doses.

RADIATION PROCESSING OF FOOD[d]

The radiation dose depends on the desired effect(s) and, for a given effect, on factors such as the type of food treated and conditions chosen for irradiation. The applied dose of radiation ought not to exceed that needed to achieve the desired effect(s). Facility design should attempt to optimize the dose uniformity ratio and to ensure appropriate dose rates. Routine dosimetry should be carried out during operation. The in-plant control of radiation doses should be monitored by national authorities in accordance with the internationally accepted procedures.

A given permissible overall average dose can be administered as a single treatment or as more than one consecutive treatment (fractional irradiation). Similarly, provided that the total permissible dose is not significantly exceeded, it is irrelevant whether a composite food contains one or several irradiated ingredients. Repeated irradiation should be used only in cases where there are technological needs for it (e.g. re-irradiation at a much higher dose for another technological purpose or if the full dose has to be applied in two or more instalments).

As long as good radiation processing practice, including use of radiation sources with acceptable maximum energies, is complied with, no health problems can be attributed to induced radioactivity in irradiated food. In any case, any such induced radioactivity is extremely low and below the present limits of detection.

The Committee recognizes that there are a number of methodological and technological requirements which need to be satisfied in order to ensure the wholesomeness of irradiated foods. They include irradiation facility design and management as well as methods to preserve the desirable properties of irradiated food. Radiation treatment of food should be carried out in facilities which are designed to meet the requirements of occupational safety, efficacy and good hygienic practice of food processing, and staffed by trained, competent and adequately protected personnel. Adequate records of all irradiation operations carried out by the facility should be kept. Where appropriate, a visual colour change radiation indicator should be affixed to each product pack for ready identification of irradiated and non-irradiated products. Facility design should permit control of

[d] See also Section III.

temperature and atmosphere during irradiation. It is also necessary to minimize mechanical damage to the product during transportation, irradiation and storage, and desirable to ensure the maximum efficiency in the use of the irradiator. In order to preserve desirable properties of irradiated food it is essential to comply with good manufacturing practice including adequate packaging and, in some cases, refrigerated storage. While comprehensive discussion of these aspects is beyond the scope of this report, careful considerations need to be paid to them. These aspects have been extensively discussed jointly by FAO, IAEA and WHO in 1981.

Moreover, radiation processing is not a system to make good the effects of prior negligent handling. Because of safety reasons, radiation processing should be applied only to foodstuffs complying with the standards of good manufacturing practice. The Committee considers it important that good manufacturing practice be observed also before food irradiation is applied as is required for other means of food preservation. Furthermore, the Committee considered labelling of irradiated food useful, although this question was not addressed in detail. The Committee noted approaches adopted so far by the Commission of the European Communities and by the Codex Alimentarius and agreed in principle with them.

RADIATION CHEMISTRY OF FOOD[e]

The Committee considers that radiation processing of food cannot be compared with the use of food additives, as there is no question of transfer of specific substances to the food. The Committee agrees with the view that food irradiation should be regarded as a process comparable to, for example, heat treatment.

The recent developments in food irradiation chemistry have contributed remarkably to elucidating the nature of the changes occurring in irradiated foodstuffs as well as the mechanisms of radiation chemical reactions in the major food components. Pulse radiolytic techniques and electron spin resonance spectroscopy are valuable methods for determining any radical intermediates produced, whereas identification and quantification of chemical changes due to irradiation are usually carried out by means of high pressure liquid chromatography and mass spectrometry techniques.

Extensive data, particularly on volatile components, show that the same constituents in different foods (e.g. carbohydrates, fats and proteins) undergo similar chemical changes. Therefore, in complex foods, the nature of the chemical changes induced by irradiation in individual food components can be considered largely the same. Other factors being equal, yields of chemical changes are determined mainly by the concentrations of precursor components and the amount of energy absorbed. Moreover, the quantity of chemical products formed by irradiation is consistenly related to the amount of water present in food. This is due to the fact that most chemical changes result from reactions of the hydroxyl radical with other food components and that water is the primary source of hydroxyl radicals in food. Other important factors are whether irradiation is carried out at ambient or low temperature and in the presence or absence of oxygen.

In spite of the several variables controlling the chemical changes and their nature, the maximum possible yields of most of the products formed by irradiation are predictable, if the composition of the irradiated food and the irradiation conditions are known. If appropriate precautions are taken to minimize changes, radiation processing of food only produces chemical products in the ppm range. For example, most (90 %) of the volatile components detected in meat are also present in food preserved by other processes (e.g. heat).

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[e] See also Section IV.

Theoretical calculations based on radiation chemistry indicate that irradiation of meat at doses up to 1 kGy yields maximum levels of total unique volatile products (not identified so far in non-irradiated food) of the order of 3 ppm, with any one of them being far below 1 ppm. The structures of the unique compounds identified so far are related to natural food constituents. Less is known about the chemical nature of non-volatile fractions of the products formed in food by irradiation.

METHODS TO IDENTIFY IRRADIATED FOODS[f]

Considerable efforts have been devoted to the development of analytical methods for identifying irradiated foods. However, at present only qualitative methods, applicable to selected food items, are available. More satisfactory methods need to be developed.

NUTRITIONAL ASPECTS[g]

As far as the nutritional aspects of food irradiation are concerned, a large amount of data show that most components of food are not significantly changed upon radiation processing. However, some vitamins (e.g. vitamins B, C and E) and the polyunsaturated fatty acids may be affected. The extent of losses of such nutrients due to food irradiation depends on several factors which include the type of food, the irradiation conditions (e.g. energy and doses) and storage conditions (e.g. temperature and presence of air). There is no evidence that technologically appropriate irradiation treatment up to 1 kGy will cause major nutrient losses in any foods, but higher doses may cause significant losses of some essential nutrients in some food. This can be prevented only by proper technological precautions during irradiation and storage. In general, nutrient losses caused by food irradiatin are unlikely to be significantly different from those induced by other methods of processing and storage. The Committee is aware that the overall importance of nutrient losses in an irradiated food also depends on the importance of the specific food to the total diet.

TOXICOLOGICAL ASPECTS[h]

A number of toxicological studies are available on irradiated isolated food components as well as on irradiated foods and feeds. Approximately 60 different irradiated food items have been submitted to toxicological investigations and about 20 food items have undergone very comprehensive toxicological trials including one or more long-term and multi-generation studies (see Annex).

The Committee felt it appropriate to discuss specifically some contradictory toxicological data that have been extensively debated. A small number of positive findings indicate that mutagenic principles can be formed under certain circumstances in food and isolated food components due to irradiation. The presence of such mutagenic substances has been demonstrated in sensitive test systems only after irradiation at high dosage, and then only if the tests are performed shortly after irradiation. No mutagenic effects have been demonstrated in foods which had been irradiated at the technologically relevant dosages and then stored or heat-treated. Presumably, this has to do with the fact that the active substances possibly formed during irradiation at the technologically relevant dosages either are not formed in biologically significant quantities or are rapidly inactivated by reaction with other food components. Similar considerations apply to previously described animal studies indicating a slightly increased incidence of polyploidy in bone marrow cells or peripheral lymphocytes upon administration of freshly irradiated wheat or potatoes.

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[f] See also Section V.

[g] See also Section VI.

[h] See also Section VIII.

The likelihood of toxicological concerns specifically associated with peroxidation of polyunsaturated fats was also addressed. The Committee has reviewed the many available reports and has concluded that the data indicated that the irradiation of foods at the stated dosages will not cause exposure of human beings to toxicologically significant quantities of products formed through peroxidation of polyunsaturated fats. This conclusion is also supported by several biochemical studies carried out to verify whether the daily administration of irradiated fats may change the ability of rodents to metabolize xenobiotic substances.

Taken as a whole, the toxicological studies do not indicate adverse health effects from dietary exposure to irradiated food.

A remarkable amount of data are also available on irradiated animal feeds. They were reviewed by the Committee who has concluded that both laboratory and commercial animals grow and reproduce normally on diets irradiated at doses up to 15 kGy.

On the basis of all the information reviewed, the Committee is of the opinion that in order to assess the safety of a food irradiated up to 10 kGy no further animal feeding studies need be carried out. This was also the opinion of the Joint FAO/IAEA/WHO Expert Committee on the Wholesomeness of Irradiated food in 1980. The Committee has not considered the specific issues relating to the irradiation of food additives and food packaging materials.

CONCLUSIONS

On the basis of all the available evidence the Committee recommends that in the context of an overall assessment of the wholesomeness of irradiated foods only those specific irradiation doses and food classes should be endorsed that are indicated as appropriate, not only from a strict toxicological point of view, but also from a chemical, microbiological, nutritional and technological standpoint. Table 1 lists the food classes and radiation doses submitted to the Committee and considered by it to be acceptable from a public health standpoint. The Committee believes that the health significance of any changes which may take place in the listed foods at the indicated radiation doses is not different from the health significance of the changes which are induced by heat treatment.

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

Table 1 : Acceptable irradiated food classes and radiation doses

FOOD CLASS	OVERALL AVERAGE RADIATION DOSE (kGy)
1. Fruits	up to 2
2. Vegetables	up to 1
3. Cereals	up to 1
4. Starchy tubers	up to 0.2
5. Spices and condiments	up to 10
6. Fish and shellfish	up to 3
7. Fresh meats	up to 2
8. Poultry	up to 7

II. INTRODUCTION

The Committee included within its terms of reference not only the evaluation of potential health aspects directly related to toxicological and nutritional properties of irradiated foods but also the possible pathogenic and food-spoilage properties of organisms surviving radiation processing of food. The Committee considered beyond its mandate the assessment of societal demands for food irradiation in comparison with other processes.

The initial research into the scientific and technological aspects of food preservation and sterilization by irradiation as a credible alternative technology was carried out in the US in the late '40s. At the same time, concern arose over the wholesomeness of food preserved in this manner and in the light of the legal approach required by the US Food Legislation it was then necessary to investigate each individual irradiated food as if it were a food additive. This being the only guide then available to national authorities and international Expert Committees, it was not surprising to find that a large number of expensive, lengthy and sometimes repetitive animal studies were being carried out in a number of countries.

To rationalize and coordinate these various efforts in a more economic fashion, the International Project in the Field of Food Irradiation was set up in 1971 as a result of an agreement between 19 interested countries under the joint sponsorship of the International Atomic Energy Agency (IAEA) and the Food and Agricultural Organization (FAO), both UNO agencies, and the European Nuclear Energy Agency, later renamed the OECD Nuclear Energy Agency. The Federal German authorities provided Host Centre facilities at Karlsruhe, the IAEA paid the salaries of the Project staff and the NEA provided the secretariat for the Project's committees. Membership soon rose to 24 countries by 1975 and remained at that level until the termination of the Project on 31 December 1981.

The objectives of the Project were essentially the carrying out of a modest research programme into methodology at the Host Centre and the coordination, including supervision, of wholesomeness testing and related studies in laboratory animals, contracted out to reputable contract laboratories on behalf of the membership of the Project. In addition, the Project undertook the collection, collation and dissemination of information concerning wholesomeness testing of irradiated foods and provided assistance to national authorities in their consideration of the acceptance of irradiated food.

During its 11 years of existence the Project issued 12 volumes of a bulletin entitled "Food Irradiation Information" and 67 Technical Reports on the various wholesomeness studies carried out under contract or performed in the laboratory at the Host Centre. Up to 3000 copies of each Bulletin and Technical Report were distributed to some 70 countries. A number of scientific papers reporting the results of some of the wholesomeness studies and also describing a newly developed methodology for assessing the genotoxicity of irradiated foods were published in the open scientific literature. Two extensive monographs entitled "Radiation Chemistry of Major Food Components" and "Recent Advances in Food Irradiation" were published in book form in 1977 and 1983.

The Project placed some 12 extensive feeding studies with contract laboratories to investigate toxicological aspects of irradiated potatoes, wheat, flour, fish, rice, spices, mango, dried dates, onions and cocoa powder in order to fulfil the requests of the 1969 and 1976 Joint FAO/IAEA/WHO Expert Committees on Irradiated Food which had assessed the clearance of the irradiation process and of the irradiated foods from the point of view of safety to health. The selection of the foodstuffs was based on a consideration of the interest likely to be accorded to the product as a staple food entering international trade, its usefulness to developing countries, and its technological and economic suitability for radiation preservation by doses in the 10 kGy range.

After 1976 a sensitive methodology was developed in the Project's own laboratory, based on simple short-term mutagenicity tests on digests of irradiated foods. These biological investigations supplemented extensive coordinated programmes of research into the radiation chemistry of food and food components carried out in some 9 collaborating specialist laboratories in the world. Data were collected on the identification and quantitative measurements of radiolytic products derived from the major components of irradiated foods and compared with the effects of conventional food processing. In this way convincing evidence could be assembled for the uniformity, predictability and ability to extrapolate radiolytic reactions within a given class of foods and also from one class of food to another. As a result of all these efforts, the 1980 Joint FAO/IAEA/WHO Expert Committee accepted the safety of the process of irradiation for the preservation of food up to an overall average dose of 10 kGy. As a consequence of this decision, the Codex Alimentarius Commission developed a General Standard for Irradiated Foods and a Code of Practice for the Operation of Radiation Facilities for irradiated foods in international trade. Thus, having achieved its objectives, the International Project was wound up.

During its existence, the International Project accumulated an extensive documentation on the wholesomeness aspects and the radiation chemistry of foods and food ingredients. It also provided a survey of all literature relating to the wholesomeness aspects of irradiated foods published since 1950 in a computerized form at the Host Centre in Karlsruhe. In this way, a focal point was created for obtaining information on irradiated foods as a service to national authorities. In addition, the Host Centre has issued since 1955 a bibliography on the preservation of foodstuffs by ionizing radiation covering the compilation and evaluation of all relevant literature. Issue No 29 appeared in September 1985. Because of the availability of original data and publications, two meetings of the EEC Scientific Committee for Food were held in Karlsruhe during the preparation of the present Report on the Irradiation of Food.

III. RADIATION PROCESSING OF FOOD

1. Doses and effects

Effects and possible applications of irradiation at different doses are shown in Table 1.

Processing with doses of radiation between 0.02 and 1 kGy can influence a variety of biological processes. For instance, it may induce inhibition of sprouting during storage (e.g. of onions and potatoes) and delay of ripening (e.g. of mangoes and papaya). As radiation doses in this range kill the insects at all stages of their lifecycle, they can also be used to control insect infestation (e.g. of wheat, rice, pulses and dates) thus providing an alternative to pesticides or fumigants. Processing of foods (e.g. fish products, chicken, strawberry, and spices and condiments) with doses between 1 and 10 kGy may be used for the practical elimination of pathogenic organisms (radicidation) and of non-spore-forming microorganisms other that viruses (radurization). The benefits of radicidation and radurization are associated with the possibility of controlling many rather common health hazards related to food-borne diseases. Many raw foods, e.g. meat and poultry may harbour pathogens of major public health concern such as Salmonella, Campylobacter and Toxoplasma. Campylobacter has been detected in most, if not all, ready-to-cook poultry. The high incidence of Salmonella in poultry and raw meat is well documented. Raw meat is likewise an important source of Toxoplasma and certain parasitic diseases like trichinosis and taeniasis. Irradiation offers a very effective means to eliminate or reduce the number of the pathogens below the minimum infective dose. Irradiation of poultry, the main source of Campylobacter and Salmonella infections in men, could substantially reduce the number of human cases of these infectious diseases of global concern. Irradiation of processed food, such as deepfrozen sea food, can effectively eliminate the risk of contamination with disease-causing agents like Salmonella, Shigella, Vibrio parahaemolyticus and enteropathogenic Escherichia coli.

Radiation processing may also effectively substitute for chemical treatment. For instance, radiation processing can be used instead of ethylene oxide in order to reduce microbial contamination in spices, dried vegetables, and thickeners.

Processing of foods (e.g. beef and poultry) with doses higher than 10 kGy may result in sterilization for commercial purposes. This process, referred to as radappertization, is also expected to reduce to some extent the number of viruses. The Committee did not review in detail the extensive data on processing of foods (e.g. beef and poultry) with doses higher than 10 kGy for sterilization purposes because the radiation conditions under which they were obtained are not relevant to the likely commercial applications of food irradiation. Effectively, therefore, this report concentrates on radiation processing at doses up to 10 kGy.

It is worth noting that not all food items are suitable for radiation processing; for instance, irradiation of milk and milk-derived products may facilitate the development of rancidity through induction of lipid peroxidation. Undesirable effects (e.g. off-flavour and odour development, discolouration and loss of texture) may occur at certain levels of irradiation, depending on the dose and the particular food. These limitations may often be prevented by control of the radiation dose and by the choice of appropriate conditions for the irradiation treatment (e.g. use of low temperature). If the development of technological research and wider application of food irradiation will occur, processing plants may have to be designed to meet specific technological requirements.

2. In-plant dosimetry

The applied dose of ionizing radiation should not be higher or lower than is needed to achieve the desired effect. Finding and applying the appropriate dose level is the key to a wholesome and technologically and economically proper application of the irradiation process to food.

Control of the food irradiation process in all types of facility (either "continuous" or "batch" type) involves the use of accepted methods of measuring the absorbed radiation dose. For all types of facility the dose absorbed by the product depends on the radiation parameters, the dwell time or the transportation speed of the product, and the bulk density of the material to be irradiated. Source-product geometry, especially distance of the product from the source and efficiency of radiation utilization, will influence the absorbed dose and the homogeneity of dose distribution.

Prior to the irradiation of any foodstuff certain dosimetry measurements should be made, which demonstrate that the process will comply with technological and regulatory requirements. Various techniques for dosimetry pertinent to radionuclide and machine sources are available for measuring absorbed dose in a quantitative manner.

Dosimetry commissioning measurements should be made for each new product or irradiation process and whenever modifications are made to source strength or type and to the source product geometry.

Routine dosimetry should be made during operation and records kept of such measurements.

Facility design should attempt to optimize the dose uniformity ratio and to ensure appropriate dose rates. Where appropriate, a visual colour change radiation indicator should be affixed to each product pack for ready identification of irradiated and non-irradiated products. Records should be kept in the facility record book which show the dosimetry, the dosimeters used and details of their calibration.

It is practical (for reasons such as the technical design of the irradiation facility) to stipulate an average dose value rather than to require that no part of the food shall receive less than a minimum, or more than a maximum dose. Taking into account the ratio of maximum to minimum dose absorbed by the product (i.e. the "dose uniformity ratio") in pilot and currently used commercial facilities, the overall average dose may result in a small fraction of the food receiving a maximum absorbed dose up to 50 % higher than this average. The overall average dose is the arithmetic mean value of all dosimeter readings in a given irradiation run. To determine this mean value, an adequate number of dosimeters must be randomly distributed in the food as it is exposed to the radiation. The number of dosimeters is considered adequate if it permits estimation of the dose distribution in each portion of the food material of different density and if the measurements are representative for all dose and density fluctuations during a usual run.

The overall average absorbed dose can be determined directly for homogeneous products or for bulk goods of homogeneous bulk density by distributing an adequate number of dosimeters strategically and at random throughout the volume of the goods. From the dose distribution determined in this manner an average can be calculated which is the overall average absorbed dose.

If the shape of the dose distribution curve through the product is well determined the positions of minimum and maximum dose are known. Measurements of the distribution of dose in these two positions in a series of samples of the product can be used to give an estimate of the overall average dose. In some cases the mean value of the average values of the minimum (Dmin) and maximum (Dmax) dose will be a good estimate of the overall average dose,

i.e. in these cases overall average dose = $\frac{Dmax + Dmin}{2}$

Some effective treatment e.g. the elimination of harmful microorganisms, or a particular shelflife extension, or a disinfestation requires a minimum absorbed dose. For other applications too high an absorbed dose may cause undesirable effects or an impairment of the quality of the product.

The design of the facility and the operational parameters have to take into account minimum and maximum dose values required by the process.

Measurements of the dose in a reference position can be made occasionally throughout the process. The association between the dose in the reference position and the overall average dose must be known. These measurements should be used to ensure the correct operation of the process. A recognized and calibrated system of dosimetry should be used. A complete record of all dosimetry measurements including calibration must be kept.

In the case of a continuous radionuclide facility it will be possible to make automatically a record of transportation speed or dwell time together with indications of source and product positioning. These measurements can be used to provide a continuous control of the process in support of routine dosimetry measurements.

In a batch operated radionuclide facility automatic recording of source time can be made and a record of product movement and placement can be kept to provide a control of the process in support of routine dosimetry measurements.

In a machine facility a continuous record of beam parameters, e.g. voltage, current, scan speed, scan width, pulse repetition and a record of transportation speed through the electron beam can be used to provide a continuous control of the process in support of routine dosimetry measurements.

3. Induced radioactivity

Electrons and photons may induce nuclear reactions if their quantum energy is above the threshold of the respective nuclear reaction. Under conditions relevant for radiation processing of food, the quantum energy of electrons is too low to cross the coulomb barrier of the nucleus. Electrons are deflected and decelerated in the electrical field of the nucleus and bremsstrahlung is produced accordingly. For the same energy dose the numbers of photons likely to hit the nucleus is lower for electrons than for photons.

Consequently gamma rays, bremsstrahlung, and electrons affect the nucleus physically through the same process : depending on the quantum energy isomeric states are induced or particles, in the majority neutrons, are knocked out. Only for a very few isotopes is the threshold for these reactions below 10 MeV quantum energy. Even in these cases the cross section (= reaction probability) is very low and additionally the abundance of these elements in food is very small. Neutrons produced by such photonuclear reactions are fast neutrons which have very small cross sections for causing other nuclear reactions. Such neutrons require to be slowed down to become thermal neutrons before they would be able to induce a measurable amount of nuclear reactions.

There is only one hypothetical process in which radiation processing of food at quantum energies below 10 MeV is able to induce measurable radioactivity. Most foods contain considerable amounts of water and water naturally contains 0.015 % deuterium. The threshold for a gamma-neutron reaction in deuterium is 2.2 MeV and consequently some fast neutrons are produced. In the unlikely case in which food consisting mainly of water were to be processed in a bulk of cubic metres the fast neutrons produced from naturally present deuterium decelerated to thermal ranges could cause further nuclear reactions. However,

the overall probability of this process is so small and the described preconditions are so unlikely to be encountered in practice, that this contribution need not to be considered further.

There remains the problem of the heavier elements which are trace components or contaminants of food. These elements have thresholds for nuclear reactions between 7 and 10 MeV quantum energy. Threshold means that the cross section (= reaction probability) is still zero for quantum energies below this value; the cross section increases to a peak value between 16 and 20 MeV and decreases at higher energies. The cross sections up to 10 MeV are very small and the induced radioactivity is of the type yielding positron emission or electron capture which always have small half-lives. This implies that any small radioactivity induced in these traces of heavy elements decays before the processed food can reach the consumer.

Taking into account this physical background and the composition of food the JECFI restricted the radiation sources for radiation processing of food to the following : (i) gamma rays from the radionuclides ^{60}Co or ^{137}Cs; (ii) X-rays generated from machine sources operated at or below an energy level of 5 MeV; and (iii) electrons generated from machine sources operated at or below an energy level of 10 MeV.

^{60}Co and ^{137}Cs isotopic sources emit radiation of maximum energy (1.5 MeV) which is too low to induce radioactivity in irradiated food. Consideration of the need to prevent induced radioactivity has lead to an international consensus that the energies of the incident ionizing electrons be restricted to values not higher than 10 MeV and those of X-rays to values not higher than 5 MeV. This is the threshold energy for nuclear transformations in the food constituents and should not be exceeded. Both experimental and theoretical considerations support this standpoint. No induced radioactivity was detected in a large number of samples of beef irradiated at a dose of about 60 kGy by 10 MeV electrons. It was estimated that any induced radioactivity, if present, must have been smaller than 0.1 % of the radioactivity normally present in food from naturally occuring nuclides (e.g. ^{40}K, ^{14}C and ^{3}H). Further investigations involving ham, pork and chicken indicated that the induced radioactivity is likely to be about one order of magnitude below the above reported maximum level, i.e. smaller than 0.01 %.

Several studies have been carried out to evaluate precisely how much radioactivity can be induced during food irradiation. The results obtained show that the amount of radioactivity produced at the irradiation conditions of 10 MeV/10kGy is below the detection threshold and approximately 100,000 folds smaller than that naturally occurring in fresh foods. The induced radioactivity originates from a small number of heavy elements present in the food. The radioactive isotopes formed have short half-lives ranging from a few hours to a few days.

In conclusion as long as good radiation processing practice is complied with, no measurable radioactivity will be induced in irradiated food and no health problems can be associated with this issue.

4. Other aspects

Comprehensive discussion of methodological/technical aspects concerning irradiation and in plant control is beyond the scope of this report. The Codex General Standard for irradiated foods and Code of Practice for the Operation of Radiation Facilities adopted in 1979 and jointly revised by FAO, IAEA and WHO in 1981, extensively discuss all technical matters relevant for food irradiation. However, in this context, it is important to draw attention to some requirements that need to be satisfied in order to ensure the wholesomeness of irradiated foods. They are as follows :

a) Radiation treatment of food should be carried out in facilities which are designed to meet the requirements of occupational safety, efficacy and good hygienic practice of food processing, and staffed by trained, competent and adequately protected personnel. Adequate records of all irradiation operations carried out by the facility should be kept. Facility design should permit control of temperature during irradiation. It is also necessary to minimize mechanical damage to the product during transportation, irradiation and storage, and desirable to ensure the maximum efficiency in the use of the irradiator.

b) In order to preserve desirable properties of irradiated food it is essential to comply with good manufacturing practice including adequate packaging and, in some cases, refrigerated storage. Additional measures may also be appropriate. For instance, as radappertization leaves most enzymes active, it is usually combined with heat inactivation or elimination of oxygen to prevent undesirable changes in properties of irradiated meats. Moreover, traditional methods of food preservation, such as curing and pH adjustment, may be appropriate to complement the effects of radicidation and radurization.

c) Radiation processing is not a system to make good the effects of prior negligent handling. For safety reasons radiation processing is only justified for products of sufficiently high initial quality.

d) If there is a technological need for it, a given dose of radiation can be administered as a single treatment or as more than one consecutive treatment (partial or repeated irradiation).

IV. RADIATION CHEMISTRY OF FOOD COMPONENTS

Like other forms of food processing, radiation processing causes chemical changes in food. The irradiation process initiates a series of reactions leading to transient radical intermediates and, ultimately, to stable new chemical products. This section deals with a number of investigations carried out to clarify the nature of radiolytic reactions and products as well as the important parameters controlling radiation-induced chemical changes in foods. The main aims of this research have been, on the one hand, the understanding of optimal irradiation conditions to minimize chemical changes and, from the other hand, the prediction of yield and nature of radiolytic products on the basis of the chemical composition of irradiated foods and irradiation conditions. It should be understood that some studies discussed in this section have been carried out under rather extreme irradiation conditions which have no practical present use in food processing.

1. Isolated fatty acids, triglycerides and phospholipids

1.1. Fatty acids

Upon irradiation in the presence of oxygen, the major transformation products of a fatty acid with n carbon atoms are carbon dioxide, hydrogen, carbon monoxide, the C_{n-1} alkane and the C_n aldehyde. Products with higher molecular weights than the parent fatty acid are also formed; they include the dimeric C_{n-1} alkane, the ketone (C_{n-1}) and alpha,alpha'-dehydrodimeric products. These results indicate that preferential cleavage of the fatty acids occur near the carbonyl bonds. Moreover, autoxidation of lipids is normally accelerated upon irradiation. Besides the products of non-oxidative radiolysis, a number of oxygen-containing products such as hydroperoxides and carbonyl compounds are formed from unsaturated fatty acids.

1.2. Triglycerides

Quantitative analysis revealed that the C_n free fatty acids and propanediol diesters are formed in high yields upon irradiation of triglycerides. A number of hydrocarbons are also formed, the C_{n-1} hydrocarbons being the most important; the yield of the C_{n-1} hydrocarbons is generally lower with the unsaturated than with the saturated triglycerides. This is explained by the preferential cleavage of the saturated triglycerides near the carbonyl group, whereas with unsaturated compounds the charge density also resides at double bond sites, thus reducing the probability of cleavage in the carbonyl region. As with free fatty acids, if triglycerides are irradiated in the presence of oxygen, autoxidation of the unsaturated fatty chain occurs and products of autoxidation as well as of irradiation are formed.

1.3. Phospholipids

Little information exists concerning radiolysis of isolated phospholipids and steroids. Recent data on dipalmitoyl-phosphatidylethanolamine, irradiated at very high doses, revealed the formation of palmitic acid, hydrocarbons, aldehydes, the symmetric ketone and esters.

2. Lipid-rich foods

Products formed upon irradiation of fat are essentially alkanes, alkenes, carbonyl compounds and alcohols. Table 2 lists the hydrocarbons formed in various fats upon irradiation at 60 kGy. Quantitative data on the hydrocarbons formed in different types of fats accorded with the values expected from the fatty acid compositions of the fat triglycerides and the radiolytic products increased linearly with radiation doses. Data on the formation of radiolytic products from fat present in meats are discussed in Section 4.

There are plenty of data showing that irradiation of lipids in the presence of oxygen would result in the dose-dependent formation of hydroperoxides and carbonyl compounds immediately after irradiation, whereas after storage hydroperoxides decay to carbonyl compounds. Moreover, if the fats contain polyunsatured fatty acids, these are likely to be lost upon irradiation under aerobic conditions. The extent of the oxidative changes can be reduced by the use of low radiation doses, by removal of oxygen before irradiation and by the presence of natural or synthetic antioxidants.

3. Isolated amino acids, peptides and proteins

3.1. Amino Acids

The aromatic and sulphur-containing amino acids are the most susceptible to irradiation and the destruction of phenylalanine is potentiated in the presence of methionine. Irradiation of free amino acids leads also to the formation of alpha, alpha'-diamino acids which, except for cysteine, are not normally found in plant and animal proteins.

3.2. Peptides

The main products of the radiolysis of peptides are ammonia, fatty acids, keto acids and "amide-like" products; diamino acids are also formed. In aqueous solutions the peptide bond exhibits an affinity towards the hydrated electron which results in the addition of the electron to the carbonyl bond of the peptide linkage.

An increase in the number of peptide bonds increases the reactivity towards hydrated electrons and the radical decay, e.g. by deamidation and main-chain scission. In peptides containing aromatic and sulphur amino acids the aromatic and sulphur residues effectively compete with the peptide bonds leading to protonation of the aromatic side groups and to sulphur radicals, respectively. With hydroxyl radicals, one of the main reactions of peptides is the abstraction of hydrogen from the carbon adjacent to the peptide nitrogen, forming "backbone" radicals. Another mechanism, particularly important for aromatic residues, involves reactions with the amino acid residues leading to side-chain radicals.

3.3. Proteins

Permanent changes in irradiated proteins include deamination, decarboxylation, reduction of disulphide linkages, oxidation of sulphydryl groups, modification of amino acid moieties, valency change of coordinated metal ions, peptide cleavage or aggregation. Radiolysis of proteins occurs through formation of ionic and free radical intermediates under the control of factors such as the structure and state of the protein and the conditions of irradiation including dose, temperature, presence of oxygen and other chemicals.

Pulse radiolysis-kinetic spectroscopy has shown that radical sites migrate within ribonuclease and ribonuclease-derived proteins and that predominantly the aromatic and sulphur-containing amino acid residues are involved as sites of transient radical intermediates. Electron spin resonance (ESR) determinations at subfreezing temperatures with meat proteins have also shown the presence of radicals on the carbon atoms of the peptide chain.

Analysis of proteins after irradiation demonstrated that a number of small molecules, such as fatty acids and mercaptans, are cleaved off. The sulphur-containing fragments are responsible for off-odour volatiles, but their formation may be greatly reduced by irradiation at subfreezing temperatures. The major part of the protein remaining after irradiation has still a macromolecular complex configuration; with globular proteins, unfolding and aggregation occurring upon irradiation, whereas with fibrous proteins degradation is more likely. The presence of oxygen prevents aggregation and leads to increased breakage of the peptide chain. Investigations of the effect of irradiation

atroom temperature on soluble proteins and LDH isozyme activities showed that soluble proteins decreased at all doses and aggregated at 50 kGy; doses of 25 kGy reduced the activity of all isozymes.

Experiments with mixtures of proteins, carbohydrates and lipids showed that, in the presence of oxygen and of unsaturated lipids, protein aggregates are formed and their formation can be traced back to free radicals of autoxidizing lipids.

Free amino acids, particularly aromatic and sulphur-containing ones, may also bind to the proteins at specific reactive sites upon irradiation, whereas cross-linking of other cell constituents such as nucleic acid with proteins seems to play a minor role. So far alpha, alpha'-diamino-dicarboxylic acids, which are formed upon irradiation of free amino acid solutions, have not been identified in irradiated proteins.

A generalized scheme of the main reactions occuring in irradiated proteins is available, but the presence of haem groups (e.g. myoglobin) strongly influences the radiation chemistry.

Although the destruction of amino acids in pure protein solutions after irradiation is evident, protein solutions containing other solutes, proteins irradiated at subfreezing temperature and dry proteins are very radiation resistant. This is shown by the fact that the enzymes causing autolysis during the storage of high protein foods cannot be inactivated at radiation dose levels used for radurization or radappertization and that only slight, if any, losses of enzyme activity occur upon irradiation of commercial preparations of proteases and pectinases to reduce microbial counts.

4. Protein-rich foods

Many data have become available through investigations on protein-rich foods.

Electron spin resonance examination of finely ground samples of enzyme-inactivated (i.e. precooked) ham, chicken, pork and beef, irradiated to 10 kGy at -80°C showed that the same free radicals are formed in each case. These ESR spectra corresponded to a collection of protein and lipid radicals stable at -80°C. As the food is defrosted the water content will cause the unstable free radicals to disappear by reacting further to form stable molecules. However, free radicals have been observed in an irradiated food based on dried fish for up to three months following the treatment and storage under dry conditions.

Electrophoretic separation of the myofibrillar proteins in irradiated ham, beef, pork and chicken showed in each case a similar pattern of degradation thus indicating that the reactions responsible for the formation and decay of the intermediates are not affected by the overall environment around the protein and lipid components.

Irradiation of different types of meats at 60 kGy resulted in the splitting of the protein and fat molecules. Products derived from proteins include methyl mercaptan, ethyl mercaptan, dimethyl sulphide, benzene, toluene, ethyl benzene, methane carbonyl sulphide, and hydrogen sulphide, whereas those derived from fats are essentially alkanes with some carbonyl compounds and alcohols. Analysis of some of the volatile products derived from different types of meats (i.e. veal, beef, mutton, lamb, pork and chicken) indicated similar yields in all cases. The amount of volatile substances produced increased linearly with the dose up to 60 kGy. A similar linear response with dose in the formation of volatiles has also been established for fish. Moreover, for beef it was shown that the yield of volatiles increased with increasing temperature in the range -185°C to + 60°C, and particularly between +20 and +60°C.

The yield-dose plots for volatile and long-chain hydrocarbons from beef samples containing 5, 20 and 30 % fat upon irradiation at 40°C with up to 45 kGy, showed excellent linear relationships. The yield of volatile and non-volatile hydrocarbons as a function of fat levels has also be demonstrated for several meats whose fat levels differ. For example, yield-dose plots obtained for hexane from ham, chicken, pork and beef irradiated at -40°C with doses 0-90 kGy, showed a marked linear dependence of hexane formed on the dose absorbed and, for each dose, on the fat content of each meat. The yields of heptadecadiene, derived from the related linoleic acid moiety by decarboxylation, are linearly dependent on the level of that specific fatty acid in the triglyceride. About six times as mucj heptadecadiene was found in chicken, in which linoleate comprises 26 % of the fat as in beef with 4 % linoleate. Similarly, the yield of propanediol diesters of palmitic acid, derived from any triglyceride having at least two palmitic acid moieties, is linearly related to the precursors abundance (weighted for the statistical likelihood of fatty acid loss). It is also interesting to note that the volatiles formed are similar for -irradiated (60_{Co}) or electron-irradiated beef and that there are no changes in the volatiles brought about by long-term storage (up to 15 months) of meats in sealed containers.

The similarity of volatile products found upon irradiation of different meats including beef, pork and lamb may explain why the typical irradiation odour has the same characteristics although the precise contribution of each class of compounds to the irradiation odour is still uncertain. It is likely that the hydrocarbons and the carbonyl and sulphur compounds all play a role in producing the typical irradiation odour detectable in meat irradiated at temperatures above freezing.

5. Isolated monosaccharides, disaccharides and polysaccharides

5.1. Monosaccharides and disaccharides

Carbonyl compounds are the most important radiolytic products of sugars.

Destruction of glucose proceeds at nearly the same rate upon irradiation of watery solutions under aerobic and anaerobic conditions, but the nature and yields of specific products formed are markedly different. Under anaerobic conditions, 2-deoxy-gluconic acid represents the main product of glucose radiolysis but, in the presence of air, formation of C-6-deoxy-products is completely suppressed and arabino-hexosulose represents the main product. These results are explained by the reaction of primary glucose radicals and oxygen, yielding peroxi radicals, which then give rise to the formation of the dicarbonyl sugars. In addition to the above-mentioned main products, a large number of other products have been identified following irradiation of glucose solutions both under aerobic and anaerobic conditions.

Degradation of fructose in aqueous solution and under anaerobic conditions has been shown to be similar to that of glucose; both deoxy- and dicarbonylsugars are formed. The irradiation of crystalline glucose leads to the formation of hydrogen, the amount of which may be correlated with the extent of destruction of glucose, and of small amounts of many monomeric products. Crystalline fructose is predominantly converted to 6-deoxy-D-threo-2,5-hexodiulose.

The radiosensitivity of the glycosidic bonds of several disaccharides in watery solution and in the absence of oxygen was shown to be largely independent on the nature of the glycosidic linkage (alpha; beta; 1-4; 1-6, 1-1 and 1-2). The radiolysis of cellobiose produced 21 different monomeric products, glucose being the most important.

Radiolysis of low molecular weight carbohydrates is considerably reduced in solutions containing also dissolved amino acids and proteins, whereas the addition of emulsified lipids is likely to exert little influence.

Investigations carried out with alpha, alpha'-trehalose indicated that the extent of protection exerted is related to the hydroxyl radical scavenging properties of the added amino acids. Cysteine protects the sugar to a greater extent than can be accounted for by its ability to compete for hydroxyl radicals, due to the fact that it can also transfer hydrogen to primary trehalose radicals (repair mechanism).

5.2. Polysaccharides

Chemical products formed in gamma-irradiated starches derived from maize, wheat, manioc, rice, potato and haricot bean include malonaldehyde, formaldehyde, acetaldehyde, dihydroxyacetone, formic acid, and hydrogen peroxide. The nature and concentration of the radiolytic products showed no marked differences among the various starches. Moreover, regardless of the source of starch, the irradiation parameters (dose, oxygen, water content, storage period) exercised similar roles in the formation of a given product.

After irradiation of maize starch at 10 kGy, 49 ppm of glycerol aldehyde, 1.2 ppm of dihydroxyacetone, and 6 ppm of 2-hydroxymalonaldehyde were formed. The quantities increased in a linear fashion with doses up to 60 kGy.

Irradiation of polysaccharides may also affect the degree of polymerisation. For instance the average number of glucose molecules per starch molecule is reduced from 1700 to 1100 on irradiation of potato starch at 1 kGy.

The formation of peroxides in starch-lipid mixtures depends essentially on lipid composition, radiation dose and duration, and temperature of post-irradiation storage; it can be prevented by appropriate concentrations of antioxidants.

6. Carbohydrate-rich foods

As fruits consist mainly of water and carbohydrates, it is expected that these two components should dominate the radiation chemistry of fruits. A model of fruit parenchyma cells, consisting of a single vacuole containing all the major fruit components in solution, was developed by Basson et al. According to this model, upon irradiation, nearly all the incident energy is absorbed by the water with production of free radicals (i.e. hydrated electron, hydrogen atom and hydroxyl radical) which then diffuse and react with the other components in a competitive manner determined by the rate constant of the reactions involved. The extent to which any of these reactions takes place is determined by well established kinetic laws and has been calculated using digital computer methods to solve the complex differential equations which describe the reaction probabilities. Chemical analysis confirmed the prediction that the radiolytic products, present in greater yield in the irradiated fruits, were derived from sugars and that yields of products derived from minor fruit constituents (e.g. proteins, malic acid, phenolics, and nicotinamide) were much lower.

The irradiation of potatoes at doses of up to 0.15 KGy with the aim of inhibiting germination increased the saccharose content between 3 and 15 fold. The saccharose content fell, however, during subsequent storage, returning to its original value after 12 weeks. Glycoalkaloids, analysed in potatoes over several seasons, did not show any significant changes with regard to irradiation dose and storage time, whereas phenolic compounds and coumarins were shown to increase with post-irradiation storage.

7. Isolated vitamins and other food components

The vitamins differ widely with regard to the ease with which they are destroyed by irradiation. Vitamins E, B_1, C, A and B_{12} are affected by irradiation, whereas other vitamins such as biotin and riboflavin are more resistant, although they can still be destroyed to a certain extent.

Vitamin E is easily destroyed by irradiation through oxidation. The thiazolium ring of vitamin B_1 is likely to be the primary site for one-electron reduction of thiamine leading to the formation of dihydrothiamine. In the case of vitamin B_{12} and its coenzyme, one electron reduction appears to cause the cleavage of the carbon-cobalt bond and to lead to formation of hydrocarbons. Dehydroascorbic acid has been identified as a primary product of vitamin C irradiation, while likely secondary products are polybasic acids including oxalic acid. Four products of vitamins D_1 and D_2 have been identified; they are all hydrocarbons arising from cleavages of the triene system of the vitamins. The side chain was unaltered, whereas the hydroxyl group of the A-ring of vitamin D was lost.

The reactions induced by irradiation of dilute aqueous solutions of DNA result in large damage (approx. 70 %) to the base moiety and to a lesser extent (approx. 30 %) to the deoxyribose moiety. So far only a few base-derived products have been identified, aalthough only in small amounts. Moreover, 2-deoxy-D-erythro-pentoic acid, 2,5-dideoxy-pentos-4-ulose, 2,3-dideoxy-pentos-4-ulose and 2-deoxy-pentos-4-ulose have been isolated from -irradiated aqueous solutions of DNA.

The radiation-induced chemistry of N-acetyl-glucosamine, a substantial component of mucopolysaccharides and glycoproteins, essentially follows similar routes as observed with glucose or ribose-5-phosphate; however, the bond between the acetamido group and the glucose molecule appears to be quite stable.

8. Seasonings and similar food ingredients

Chemical changes induced by irradiation in spices are minimal.

No changes in the flavour or content of essential oil were observed in ground paprika, black pepper and cumin which were sterilized with a radiation dose of 10 kGy. With caraway and cardamon, the yield of essential oils was slightly reduced upon irradiation at 10 kGy, but the composition of the oil and the fatty acid composition of the lipids were unchanged. Other data indicate that no change could be observed in the piperine content of black pepper at a dose of 18 kGy.

Irradiation of paprika at 0-22°C with doses of 5-50 kGy and subsequent storage for 6 months had practically no effect on the carotenoid content. In some spices radiation treatment with 5 and 15 kGy affected the relative concentrations of some fatty acids and reduced the proportion of some unsaturated fatty acids.

No change in quality could be detected by sensory means in nutmeg, marjoram, thyme, cinnamon following irradiation at doses of 5 and 10 kGy; however, the quality of finely-chopped orange peel and lemon peel and fenugreek was reduced.

9. Analogy of chemical changes induced by irradiation, heat, and other methods of food processing

A very important issue that needs clarification is whether the many chemical changes induced by radiation processing of food (see Sections IV.1 - IV.8) are specific in nature or are similar to the number of chemical changes induced by other methods of food processing. In particular the comparison of chemical changes induced by radiation and heat processing of food is very appropriate as both methods may be used to ensure the destruction of microorganisms in food and to prolong the shelf-life of food.

Available evidence indicate that properly applied irradiation is by no means more destructive than heat processing of food and that most chemical changes observed in irradiated foods also occur in heat-treated foods.

9.1. Studies on food products

Thermal decomposition products of fats upon deep frying include n-alkanes, alkenes, free fatty acids, esters, ketones, lactones, decarboxyl acids and esters, and cyclohexanes. Table 1 shows that most of the hydrocarbons formed in different types of fats as a result of irradiation at 60 kGy are also produced upon heating at 170°C for 24 hours. However, characteristic hydrocarbons have been identified for both thermal decomposition (i.e. cyclohexanes) and irradiation (i.e. tetradecadiene and heptadecadiene).

About 100 different volatile substances have been identified in boiled beef and about 175 in boiled chicken including benzene and alkyl benzenes. Moreover, sulphur compounds such as mercaptans, disulphides and hydrogen sulphide have been identified among other compounds, in canned beef. Parameters such as pH value, solubility of proteins, total number of sulphydryl groups and SDS-polyacrylamide gel electrophoresis patterns, have been investigated in irradiated and gently heated meats. Effects seen in beef and pork at the highest irradiation dose (i.e. 50 kGy) were similar to those found after heat processing at temperature not higher than 70°C. Moreover, relatively stable free radicals were found in kamaboko, a fish meat cake, not only after irradiation treatment but also after heating.

Carbohydrates are also known to undergo major changes under some cooking conditions. For instance carbohydrates react with proteins to form polymer compounds which are responsible for the brown colouration (e.g. the formation of crust in baking). The production of carbonyls (which are the most important radiolytic products of sugars) from mangoes was 5 folds higher in the canned samples than in the irradiated samples, whereas no significant difference was observed with papayas. Furthermore, a comparative study of the influence of gamma-irradiation and thermal sterilization on some components of carrot pulps showed no effect of irradiation (10 kGy) on total lipids, even after 6 months storage. Lastly, heating maize starch in suspension at 140°C for 30 minutes produced a change in viscosity greater than that observed after irradiation at 1 kGy, but smaller than the change induced by a radiation dose of 15 kGy.

9.2. Studies on isolated food components

Determinations were carried out in gamma-irradiated maize starch of glyceraldehyde, dihydroxyacetone and 2-hydroxymalonaldehyde. The amount of these three products formed by irradiation at 10 kGy were comparable with those obtained on heating at 125°C for 1 hour.

In one investigation with tricaproin, the compounds produced by heating for 15 hours at 270°C were compared with those formed by irradiation at 60 kGy. For both treatments, the products included a series of n-alkanes and 1-alkenes, the free fatty acid and its methyl ester, an aldehyde of the same carbon number as the parent fatty acids, the diglyceride, and alkane- and alkanediol-diesters. The compound produced in the greatest quantity in both cases was the free fatty acid and the major hydrocarbons were the C_{n-1} alkane and the C_{n-2} alkene. On the other hand, the relative quantitative values of the compounds produced from each treatment were different. The amount of the major alkane (pentane) produced by irradiation was nearly twice that formed by heat, while the major alkene (1-butene) was produced in greater quantities by heat than by irradiation. Moreover, the propanedial diesters obtained from heating were produced in lesser amounts than the propenediol diesters, but the reverse quantitative ratio was observed following radiolysis.

In addition to the production of volatile compounds, heating and irradiation of fats result in the formation of several types of dimeric and polymeric compounds. The structure of the dimeric compounds identified in heated methyl oleate is quite similar to that of the dimers produced by irradiation of potassium oleate.

When amino acid-fatty acid ester mixtures were heated at 250°C for 1 hour, a number of interaction products, including amides, nitriles, pyrroles, amines and pyridines, were formed.

Few or none of these interaction products could be detected and those which were identified were present only in very low yields, even upon irradiation at very high doses (250 kGy).

10. Unique radiolytic products

A number of investigations with high radiation doses on high-protein foods as well as on numerous model systems, show that radiolysis yields may be characterized as generally increasing linearly with absorbed dose. In addition, based on the energetics of ion pair products, the yield of new species formed (Radiolytic Products-RP) can be calculated from the following expression :

$$\text{Yield (in mmol/kg)} = \text{Dose (krad)} \times G_T \times 10^{-3}$$

where G_T is the number of molecules formed or destroyed per 100 eV absorbed.

It has been shown that G-values determined from the irradiation of individual compounds in solution, or from the irradiation of simple mixture (model systems), can be used to predict the total G-value in the actual food matrix.

The utility of G-values for estimating yields in irradiated foods is enhanced by the discovery that individual food components tend to produce the same radiolysis products (RPs) when isolated, or when occuring as natural components of complex foods. Expected cross-over RPs are thus minimal; for example, the reaction between lipid-derived and protein-derived free radicals are found to be limited by reactions occuring across interfacial regions between tissue phases in meat. This apparent "compartmentalization" of food components considerably restricts the spectrum of possible RPs likely to occur within or across classes of foods. Thus, foods of similar chemical composition, irradiated under similar conditions, will contain RPs derived from common precursors and such irradiated foods may reasonably be viewed in a generic sense.

For purposes of estimating the total levels of RPs in food, a value of $G_T = 1$ has been selected. The results noted above, as well as those from the Natick Laboratory, suggest that if food irradiation practices result in an organoleptically acceptable product, the actual G_T will be adequately characterized by this value. In practice, with various foods and conditions, this factor may at times be greater or less than one, but current information supports unity as a reasonable assumption. Variations of G_T of plus or minus 100 % should not significantly alter the arguments based on an assumed value of G = 1. Therefore, as indicated by the above equation, a dose of 10 kGy will yield 1 millimole of total RPs per kilogram of irradiated food. Assuming an average RP molecular weight of 300, one kilogram of food irradiated at 1 kGy will contain 300 mg of newly formed chemicals. As most of these radiolytic products are also present in non-irradiated foods, the yield of products not found in non-irradiated foods (Unique Radiolytic Products -URPs) is much smaller than that of total RPs. Moreover, the true extent of the dietary "uniqueness" of URPs is somewhat tenuous, due largely to the paucity of information on the composition of both processed and unprocessed food at the parts per million level. It is quite possible that radiolytic components at the present classified as unique to irradiated foods also occur in foods which have been processed by conventional thermal methods. Examination of the most complete set of available data on RPs in food will serve to illustrate and document the significance of distinguishing between total RPs and URPs. The U.S. Army's high-protein food sterilization programme provides detailed analysis of volatile species identified in raw beef irradiated (in vacuo at about -30°C) at 50 kGy. These volatiles consist of a nearly homologous series of 65 RPs (in concentrations of 1 to 700 g per kg) derived primarily from the radiolysis of the triglycerides from the beef lipid fraction. Of the 65 volatiles, 23 were also identified in the thermally sterilized control, so that 42 were unique to the irradiated product (URPs). However, of

these 42 URPs only six could not be identified in the volatile fractions of other non-irradiated foods. Thus only some 10 % of this particular subset of RPs (the 65 volatiles) are in fact URPs. The structures of these six URPs are typical of the molecules identified as occurring in other food volatiles, and are similar to natural food constituents.

From the above considerations, it is reasonable to assume that at least looking to the volatile species of raw beef the URPs constitute 10 percent or less of the total radiolytic product yield. Table 3 shows the expected quantity of total URPs at various radiation doses.

These conclusions are based on a series of RPs which happen to be associated with a volatile fraction of irradiated food. The question is if they do also typify the relationship of non-volatile RPs and URPs to one another, and to the fraction of RPs which are constituents present in non-irradiated foods.

V. METHODS TO IDENTIFY IRRADIATED FOODS

INTRODUCTION

It is of importance for inspection purposes to be able to demonstrate whether a food has been treated with ionizing radiation or not. For this to be achievable with any certainty, it is necessary that a radiation-specific change takes place in the food and that this change is measurable. However, those changes which take place after irradiation in many cases resemble very closely the changes which also take place as a consequence of other treatments. The methods of measurement which can be used in the course of inspection are in many cases, therefore, not based on really radiation-specific changes. Many investigations have been carried out in an effort to design reliable methods for detecting whether or not a food has been irradiated. Attempts have been made to apply physical, chemical, microbiological and other forms of measurement.

Examples are given below of a number of methods which are able to identify differences between irradiated and non-irradiated foods, although in most instances no investigations have been made to demonstrate the reliability of the method in practice, i.e. investigations which demonstrate the degree of reliability on the basis of the results of the analysis with which it can be claimed that a food has or has not been irradiated. In most cases the methods of measurement, which relate to individual foods, cannot presently be regarded as suitable methods of inspection, but rather as principles. After an eventual, and presumably labour-intensive, further development phase and standardisation, these methods could form the basis for laboratory control. Until now it has not been possible to measure the actual radiation dose to which the foods have been exposed; it is only possible to identify the radiation treatment or to give a very rough dose estimation. No single general method for all foods is available which may be used to demonstrate whether foods have been irradiated. For some dry foodstuffs, two measurement techniques are well developed for the identification of irradiation and can be used in practice in the near future. Only only methods which seem likely to be of some practical use are discussed below.

1. Physical measurements

1.1. Measurement of free radicals

Because of the short life of the free radicals following irradiation their measurement by ESR is suitable only for some foodstuffs to demonstrate that a food has been irradiated. The life of the radicals is affected by the presence of water and is of the order of seconds or minutes when water is present in liquid form, as in meat, for example. In the case of dry products they can be measured for days or even months following irradiation. Consideration has been given to whether ESR measurement can be used with ground paprika. Differences between irradiated paprika could be observed for the first 2-3 weeks. 79 % of the radicals disappear during the first 150 hours of storage, after irradiation at 10 and 50 kGy. When evaluating the ESR result, it is also necessary to take into consideration the fact that it was possible in the case of ground paprika to measure an ESR signal originating from the energy absorbed during the grinding process. Free radicals whose presence is demonstrated by ESR measurement cannot be regarded as a unique feature of irradiation.

In some new experiments conducted on black pepper, sage and dehydrated onions, irradiated with 1, 3, 10 and 30 kGy at 25°C, free radicals were detected using ESR spectroscopy. However, upon storage of irradiated spices the free radicals decayed within 4 to 5 days storage at 25°C after irradiation. The free radicals also decayed rapidly in contact with water or salad dressing, substances likely to be encountered when using irradiated spices as food condiments.

It has been claimed recently that ESR provides an excellent method for the identification of irradiated foods containing bone or calcified cuticle, even in the absence of unirradiated controls. It also shows promise for identifying irradiated strawberries.

1.2. Measurement of conductivity

The conductivity of potatoes which have been irradiated at a dose of 0.05 to 1 kGy to impair their germination is lower than that of non-irradiated potatoes. This reduction is, however, subject to variations between one variety and another and has been assessed as being unsuitable for use in conjunction with inspection. However, if the conductivity observed immediately after the electrode is inserted is compared with the conductivity after 180 seconds, a more reliable opportunity will be provided to demonstrate the effect of irradiation. The fall in conductivity over 180 seconds is greater in the case of non-irradiated potatoes than in the case of irradiated potatoes. The fall in conductivity over 180 seconds is reduced as the dose increases. This phenomenon is associated with damage caused to the cells in the potato, since the repeated insertion of the electrode into the same measurement hole does not exhibit this fall in conductivity.

New experiments, published 1982 showed better results. Measuring the impedance was found to be a highly reliable and practical technique for identifying irradiated potatoes. Impedance was measured by puncturing a potato tuber with a steel electrode and passing a 3 to 5mA alternating current through it. The technique allowed not only differentiation between unirradiated and irradiated potatoes but also an estimation of the irradiation dose for up to six months after irradiation, independent of the potato storage condition.

1.3. Thermoluminescence measurements

Thermoluminescence dosimetry is a well established measuring method in the field of radiation protection. Thermoluminescence measurements were also elaborated to determine whether spices have been irradiated or not. Luminescence intensities of more than 20 different spices were examined and observed to depend on radiation dose (0-10kGy) and storage time after irradiation. The luminescence effect from radiation treatment differs from spice to spice. Intensity increases in samples treated with 10 kGy vary between a factor of 1 (no effect) and approx. 1000 in comparison to untreated samples. In most cases it was possible to identify radiation treatment with 10 kGy, if irradiation occurred 2-3 weeks prior to the examination. In many spices, an identification is possible even as late as half a year after irradiation (Table 4). One problem of the method is, that the luminescence intensities after irradiation can vary over a broad range using the same type of spices from different producers. But in spite of that, the method is not far away from application in practice.

2. Chemical measurements

2.1. Chemiluminescence measurements

Various saccharides, amino acids and inorganic salts have been used for dosimetric measurements of ionizing radiation applied to solids. When these substances are brought in contact with water after irradiation, light is emitted in the form of a short impulse (chemiluminescence, lyoluminescence). The amount of light can be correlated to the radiation dose. Identification is based on the reaction of stable radicals in dry solids or of the irradiation-induced oxidation products during the dissolution process. The light yield can be increased by adding a photosensitizer (e.g. luminol) to the solvent. If the irradiated substance is insoluble in the luminol solution, the reaction only occurs at the surface of the substance.

During the last years, chemiluminescence measurements were also elaborated to determine whether spices had been irradiated or not. Luminescence intensities of more than 20 different spices were examined and correlated with radiation dose (0-10 kGy) and storage time after irradiation. The luminescence effect from radiation treatment differs from spice to spice. Increases of the intensity in samples treated with 10 kGy vary between a factor of 1 (no effect) and approx. 1000 in comparison to untreated samples. In most cases it was possible to identify radiation treatment with 10 kGy, if irradiation occurred 2-3 weeks prior to the examination, but also after some month the radiation treatment is still measurable for a lot of spices using the chemiluminescence method.

Thermoluminescence proved to be more productive compared to chemiluminescence. Thermoluminescence made it more frequently possible to identify radiation treatment after many months (Table 4). A combined or simultaneous use of both examination methods assures a rapid identification of radiation treatment in most of the examined spices. With the exception of garlic, onions, white and black pepper, this is possible even after a prolonged period of storage (Table 4).

2.2. Hydrogen measurement

Hydrogen gas can be used in the case of foods packed in metal cans to determine if the foods were irradiated. If there is more than 2% hydrogen gas present, the food has been irradiated.

2.3. Measurement of volatile hydrocarbons

A high-protein food sterilization programm in the USA provides detailed analysis of volatile species identified in raw beef irradiated (in vacuo at about -30°C) at 50 kGy.

3. Biological measurements

3.1. Changes in microflora

Consideration has also been given to whether a change in the microflora provides a possible method of inspection. Experiments have been performed on strawberries in an attempt to discover a microorganism which is particularly sensitive to irradiation and which, by its absence after the irradiation process, could be used as an indicator to show that irradiation has taken place. One possibility in this respect is a Gram-negative, rod-shaped bacterium.

One example is the work, published 1977 in which a system was elaborated to determine whether strawberries have been irradiated, using 3 criteria, namely the number of Enterobacteriaceae, the percentage of yeasts in the total microflora (or total absence of microorganisms) and the number of Pseudomonas. The higher the number of Enterobacteriaceae and/or Pseudomonas, the lower the probability that the strawberries have been irradiated. The higher the yeast percentage, the more the conclusion is justified that irradiation has taken place. The same holds true for total absence of microorganisms. By combining results for the 3 criteria an identification scheme was drawn up that would have led to 189 correct decisions (92.2%) on 205 samples (102 irradiated with 2 kGy, 103 unirradiated).

Many experiments have been carried out while studying the prolongation of the shelf-life of prepacked fillets of cod (Gadus callarias) and plaice (Pleuronectes platessa) and cooked shrimp (Crangon crangon) by irradiation at a dose of 1 kGy. Colonies of Pseudomonas putrefaciens, Photobacterium spp. and 'typical shrimp spoilling' bacteria (presumably Alteromonas spp.) can be differentiated. These and several other species that are involved in the spoilage of unirradiated fish and shrimp are eliminated by irradiation. In irradiated fish and shrimp Moraxella spp. predominated during the whole storage

period. Their colonies typically differ from the colonies of the former species. The predominance of Moraxella-type colonies on the plates in combination with the absence of colonies of the radiosensitive species mentioned above is indicative of irradiated samples.

3.2. Inhibition of germination

In the case of onions attempts have been made to utilize the desired effect of germination inhibition as a mean of checking whether or not irradiation has taken place, by making onions germinate under standardised conditions. Germination was found to be absent in 75 % of the onions after irradiation at a dose of 0.05 kGy, and inhibited germination was noted in 90 % of the onions after irradiation at 0.1 kGy.

VI NUTRITIONAL ASPECTS

Two approaches have been adopted to evaluate the nutritional quality of irradiated foods. The first one is based on chemical analysis with emphasis on quantitative evaluation of irradiated food constituents of major significance, whereas the second approach relies on animal short-term trials. In vivo tests dealt with in this section do not include toxicological studies that also may provide some information on the nutritional value of irradiated foods; these are discussed in Section VIII.

1. Foods of animal origin

As indicated by extensive analytical studies available, losses of some vitamins, particularly vitamin B_1 (thiamine), and of polyunsaturated fatty acids are the nutritionally significant effects of irradiation on animal foods (Table 5). Losses of essential nutrients increase with radiation dose and temperature as well as the presence of oxygen during irradiation and post-irradiation storage. For instance, the high loss of vitamin B_1 caused by radiation processing of food can be largely prevented if the temperature is lowered. If meat is irradiated in a deep-frozen state and stored as such, loss of vitamin B_1 is only 15 % upon irradiation at doses as high as 45 kGy. Moreover, irradiation of meat at -40°C led to only minor and acceptable change in meat proteins and no free radicals persisted. There were no significant losses of amino acids and little structural alterations of proteins took place. Furthermore, the loss of vitamin B_1 and of other essential nutrients can be largely prevented by excluding oxygen, for example by packing under vacuum or under a nitrogen atmosphere. The use of radiation doses up to 10 kGy for radurization of meat and poultry does not result in major chemical changes particularly if vacuum packaging is used to reduce oxidative changes and several studies indicate that lipid oxidation is not a problem in fish radurization under vacuum packaging.

The above mentioned conclusions are also supported by the results of in vivo investigations. In fact, meat irradiated up to 70 kGy showed in rats similar digestibility, biological value, net protein utilization and amino acid composition as untreated meat. Similarly, although lipids are oxidised, degraded and decarboxylated, no effect on the digestibility for man of pork meat and pork fat treated with 28 kGy and stored for 1 year was detected. However, lard irradiated at 56 kGy, was more slowly absorbed by dogs than untreated material. Chicken meat, irradiated at 6 kGy, stored for 6 days at 5°C and then cooked showed no difference in lysine availability or protein efficiency ratio when compared to untreated poultry meat. Lastly, when mackerel was irradiated with doses 1-45 kGy, filleted, ground and stored at -22°C in plastic bags, irradiation had no adverse effects on digestibility, biological value and net utilization of proteins.

Some investigations have also been carried out on casein and model mixtures containing casein. Casein and mixtures of casein with glucose or starch were sterilised by irradiation or heat. Irradiation did not cause any decrease in protein utilization and digestibility, whereas heat sterilization resulted, in the presence of glucose, and in a significant reduction of protein digestibility and in net protein utilization. Irradiation at 50 kGy of protein-unsaturated lipid mixtures and storage under normal aerobic conditions generally resulted in a reduction of net prktein component which is probably due to the formation, through lipid oxidation, of carbonyls capable of reacting with proteins and destroying lysine and other amino acids. However, when a casein-unsaturated fat was irradiated with 50 kGy in the absence of oxygen, stored for 12 weeks and then fed to rats no reduction in net protein utilization was found. Similarly, using a saturated fat, irradiated and stored under aerobic conditions, no effect on net protein utilization was noted.

2. Foods of plant origin

Chemical investigations showed that radiation processing induced only minor changes in the nutrient compositions of a variety of plant foods (Table 6 and 7). In the case of fruits, vegetables and tubers (Table 6) the nutritionally significant change more commonly detected is a reduction of vitamin C and carotene. The loss of vitamin C was particularly important in oranges and orange juice. It should be pointed out that many conflicting results are available on the loss of vitamin C after irradiation due to methodological differences, to vitamin C lability and to the fact that some authors have measured ascorbic acid but not dehydroascorbic acid that may be formed from ascorbic acid upon irradiation and which is also biologically active. In the case of cereals and pulses (Table 7), apart from the loss of vitamins B and E, no remarkable chemical changes have been shown so far.

Several in vivo investigations are also available on irradiated foods of plant origin. Macacar beans irradiated at 1 or 10 kGy and stored for 6 months showed a decrease in the protein efficiency ratio that could not be explained by chemical analysis, whereas irradiation of dry field beans increased nitrogen retention by chicks. Moreover, sorghum and millet irradiated at 0.2 kGy did not show any negative nutritional effects upon feeding to rats. Experimental studies on many animal species showed that the nutritional quality of diets containing up to 18 % dry weight irradiated potatoes is comparable to that of diets containing equivalent amounts of non-irradiated potatoes. These in vivo findings are in agreement with the fact that carbohydrates, although depolymerised and oxidatively degraded by irradiation, maintain their biological availability. The level of vitamin E, that is particularly important in oil produced from plants, may be reduced by about 50 % upon irradiation of the oil at 1 kGy. The loss of vitamin E can be partly prevented by excluding oxygen during irradiation and storage.

3. Animal diets

Many analytical studies available on laboratory and commercial diets show that vitamins A, C, E, K and folic acid are reduced after irradiation at and above 25 kGy. Lysine and other essential amino acids are only slightly affected. Peroxide levels were increased 6 to 8 fold in dry cat food by 25 kGy; however, in vacuum-packaged cat foods the increase was only 3 to 4 fold. When a semi-purified chick diet containing 10 % soya-bean oil was irradiated at 6, 30 or 60 kGy, its crude fat content decreased at the highest level; the peroxide concentration was more than doubled immediately after irradiation at 60 kGy and after 1 and 2 weeks of storage was 347 and 960 meq/kg and 1966 and 1335 meq/kg respectively, compared with values of 55 and 82 meq/kg fat in control diets.

Several nutritional studies have been carried out on irradiated feedstuffs (e.g. soya-bean meal, pea-oat mixture and fish meal) with different animal species (e.g. poultry, sheep, pigs and calves). All animals appear to thrive normally on irradiated diets where the dose does not exceed 15 kGy, whereas diets irradiated at 25 kGy require supplementation with vitamins. Chicks given an irradiated diet containing 10 % soya-bean oil showed reductions in feed consumption and feed efficiency at 60 kGy. Irradiation of the diet without the oil supplement had little effect on the growth. Digestibility and metabolizable energy were also reduced on irradiation at 6, 30 or 60 kGy and chicks on these diets showed marked dilatation of the small intestine and liver as well as increased red cell fragility. These phenomena also occurred in chicks given a diet containing the highly oxidized oil. A slight increase in digestibility and a slight decrease in the biological value of a single cell protein occurred when doses of up to 40 kGy were applied, but net protein utilization was not affected.

4. Analogy of nutritional changes induced by irradiation, heat and other methods of food processing.

It is well known that several vitamins are highly sensitive to heat, oxygen and/or light. In particular vitamin B_1 is highly unstable to heat, vitamin C and E to oxygen, and vitamin B_2 to light. This explains why considerable losses of some vitamins may occur upon food storage and cooking regardless of irradiation. For instance the vitamin C content of apples is halved after one week at room temperature, but only after three months at 4°C. Moreover the content of vitamin C in potatoes is halved in about 9 months, even if the temperature is kept at 0°C, and that in peas is reduced by more than 50 % upon cooking. Heat treatment was more detrimental to ascorbic acid in mangoes, papayas and litchis than irradiation, whereas in orange juice losses of ascorbic acid were 6-8 % after heating at 45-55°C and 20-70 % after irradiation at 2.5-10.0 kGy. In the above-mentioned fruits, carotene was not greatly influenced by either process, but in papayas the thermally-treated samples showed a 10-fold greater loss. There was no marked difference in the niacin and riboflavin contents of mangoes after irradiation at 4 kGy or heating at 100°C for 12 min., whereas 12 % more thiamine was destroyed after heating. Moreover, the loss of thiamine upon heat sterilisation was about 70 % and was similar to that observed upon irradiation at high doses (20-30 kGy). Although they may be increased by irradiation, losses of thiamine upon storage may be considerable regardless of irradiation (for an example see Table 7, number 5).

Lastly a comparison of losses of five amino acids (i.e. methionine, lysine, arginine, phenylamine, and leucine) and vitamin A in heat or radiation sterilized animal feed indicated that autoclaving is a far more destructive method than irradiation.

VII MICROBIOLOGICAL ASPECTS

Death of microorganisms from exposure to ionizing radiation is logarithmic in nature. Thus, a constant fraction of the population will be killed at equal time intervals regardless of the total numbers.

The higher the initial viable number of bacteria in food, the higher is also the final number in the irradiated foods if the irradiation dose applied is not sufficient to reach the irradiation death point of the microbial population.

It follows that good hygienic manufacturing practice for food production cannot be substituted by irradiation, a philosophy well known in other methods of preservation, e.g. heat preservation.

1. Impact of intrinsic and extrinsic factors in food

The efficacy of food in reducing the microbial load depends, beside the total viable count and the composition of the microflora, on a number of other factors, the most important of which are the composition, pH and water activity of food, the atmosphere or gaseous environment and temperature during irradiation, and the storage conditions after irradiation.

The more complex the food, the greater the competition of the components of the medium for the free radicals and activated molecules produced by the radiation, thus indirectly sparing the microorganisms. It has been shown in cured meat that the bacterial spores are sensitized towards irradiation. This is similar to a sublethal heat treatment which in combination with other methods of preservation, e.g. reduced water activity and pH, might make a food microbiologically stable, the so-called "hurdle effect". Irradiation may also form part of this effect. Application of irradiation to food makes it possible to reduce or completely omit the effect of other methods of preservation and still have some "hurdle effect" in the food. This is especially beneficial in cured meats, where reduction or omission of nitrate or nitrite in curing salts or curing brine will reduce the amount of carcinogenic nitrosamines formed.

The bacterial spores are practically unaffected by irradiation in the pH range 5-8. Below pH 5, increased sensitivity is observed.

Elevated temperatures during the irradiation enhance the irradiation sensitivity of the microbes. On the other hand irradiation of foods in the frozen state increases the radiation resistance of many vegetative bacteria by a factor of about 2. For certain Pseudomonas and Acinetobacter an increase by a factor of 6.7 has been noted.

It is a well-established fact that the presence of oxygen increases the lethal effect of irradiation on the microbial cell, and oxygen present during post-irradiation storage can enhance radiation inactivation of microorganisms.

Most vegetative bacteria are more sensitive in high-moisture environment (high water activity) than in a dehydrated microclimate, similar to what is known for heat treatment.

Microorganisms sublethally injured as the result of irradiation are more fastidious in their growth requirements and also more susceptible to unfavourable microclimatic conditions in the food during storage.

2. Beneficial microbial effects of irradiation

Irradiation of food serves two important purposes :
1) To reduce or eliminate the spoilage microflora and hence to improve the keeping quality.
2) To reduce or eliminate the load of pathogenic bacteria entering the food chain.

A reduction of the total load of bacteria also reduces the number of pathogens, depending on the composition of the microflora. Lowering the number of pathogens in food might well make the food safe for consumption even though pathogens are not completely eliminated, because their number has been reduced below the minimum infective dose.

2.1. Effect on bacteria

Radiation sensitivity of microorganisms differs with species and even with strains, although the difference in strains of single species can usually be ignored for practical purposes.

There is good correlation between the effect of irradiation on bacteria and heat treatment, with only some minor differences in detail. The most heat sensitive bacteria are also amongst those which are most readily inactivated by irradiation. The differences in irradiation resistance among gram-negative and gram-positive bacteria as well as spore-formers, are given in Table 8.

The gram-negative bacteria, including common food spoilage organisms such as Pseudomonas and most Acinetobacter as well as enteric species including pathogens such as Salmonella, Shigella, Campylobacter, and Yersinia enterocolitica, are generally more sensitive than bacterial spores which are more resistant, and Micrococcus radiodurans is exceptionally resistant.

There is little doubt that irradiation processing of food and feed can reduce the load of pathogens entering the food chain, thus complementing the other hygienic measures for the control of food-borne diseases. For instance, the public health problem predominant by far in poultry is the presence of Salmonella and Campylobacter, which can be reduced not only through irradiation of refrigerated or frozen chicken, but also through irradiation of animal feeds. There is unanimous agreement on the fact that the most efficient way to remove Salmonella from ready-to-cook poultry is irradiation. Moreover, poultry is the most important source of Campylobacter infections in the industrialized part of the world.

The importance of enteric Campylobacter infection has in many parts of the world superseded Salmonella infection in man. Irradiation is also recommended as the most efficient way to eliminate Campylobacter. The radiation resistance of Campylobacter is much lower than that of Salmonella. In ground beef, D-values for Salmonella species, different Y. enterocolitica types, and three Campylobacter jejuni strains, have been shown to be 0.55-0.78 kGy, 0.1-0.21 kGy, and 0.15-0.15 kGy, respectively. The authors concluded that a dose as low as 1 kGy reduced the number of Salmonella by approx. 1.3-1.8 log cycles (factor 20-65). Y. enterocolitica and C. jejuni would be almost totally eliminated with this dose because of their very low radiation resistance.

Pork meat and meat products containing pork are in some countries an important source of human yersiniosis. The very low irradiation resistance of Y. enterocolitica offers good possibilities for its elimination from food as a result of irradiation.

Irradiation of fish and seafood has a good potential for the control of some of the most important pathogens associated with these foods, e.g. Vibrio parahaemolyticus, NAG cholerae, Salmonella species and Shigella. A large outbreak of shigellosis in the Netherlands in 1983/84, caused by imported frozen shrimps contaminated with Shigella flexneri 2, caused the death of 14 persons.

It has long been recognized that raw milk and raw egg products should be processed for safety before reaching the consumer. This can easily be achieved by a combination of surface heat treatment and/or irradiation of the final packaged food.

The efficacy of irradiation in reducing the total number of bacteria in food is well-established. While high-dose irradiation (radappertization) aims at achieving commercial sterility of the food, it is quite clear that low-dose irradiation treatment, radicidation or radurization, has selective effect on the microflora of the treated food due to the species-dependent irradiation resistance.

Among organisms surviving low-dose treatment are spores of Clostridium and Bacillus species. Clostridium botulinum represents a special problem because of the high radiation resistance of its spores. However, extensive investigations carried out on low-dose irradiation of fish and fish products, aiming at preventing the possible formation of toxins, showed that this process is technologically feasible for ensuring the safety of the product. Irradiation must be carried out under good manufacturing practice including the avoidance of excessive initial microbial loads and carefully controlled post-irradiation handling and storage conditions. The hazard of possible toxin production cannot be eliminated through the additional use of salt alone.

Some Moraxella-Acinetobacter species, which are gram-negative Coccobacilli, have been found to survive radiation processing in fish as well as in beef and poultry. It is worth noting that Moraxella-Acinetobacter species have also been isolated from various types of unprocessed foods, e.g. beef, poultry, dairy products, fish, and vegetables. Other radiation resistant bacteria (e.g. Micrococcus radiodurans) have been identified, but generally they do not cause spoilage or disease.

2.2. Effect on fungi

The radiation resistance of moulds is of the same order as that of the vegetative bacteria except for the most sensitive ones (Table 8). Moulds are potential mycotoxin producers in food of plant origin. In these cases, control of post-irradiation storage temperature is an important preventive measure. For instance, in grain aflatoxin-producing Aspergillus strains present a possible hazard, but they do not grow and form toxin below 10°C in moist systems or even below 20°C in systems with a low water content.

The yeasts are distinctly more resistant than the moulds, showing resistance like that of the more resistant bacteria. Extremely irradiation resistant moulds and yeasts, as is the case with Micrococcus radiodurans among the bacteria, have not been observed.

2.3. Effect on viruses

Viruses have been reported to be highly radiation resistant under laboratory conditions. However, it is expected that irradiation will reduce to some extent the number of infective virus particles up to 10-fold for a dose of about 5 kGy, which is better than refrigeration which tends to preserve them. The significance of viruses in foods is still being disputed, and there is no specific requirement that viruses should be absent from food in which they are not able to multiply. Viruses are readily killed by any heat treatment.

2.4. Other effects

Irradiation is a useful alternative method of preservation in some cases where traditional treatment of foods has led to formation of cancerogenic compounds. Thus, ethylene oxide treatment of spices and other dry ingredients to be used in the manufacturing of foods to

reduce the bacterial load, especially of the spores, has given rise to public health concern. For this reason the use of the compound has actually been banned in many countries.

Although the predominant microflora in spices are spores, high doses of irradiation can be applied, since the types of organoleptic changes which occur are of no or only minor importance in spices which are only used at levels of one per cent or less in foods.

The U.S. Army Natick in co-operation with the USDA has found that irradiation processing of cured meats may permit a reduction in nitrite use. According to that research it was established that : 1) irradiation destroyed residual nitrite; 2) irradiated bacon cured with reduced nitrate (20-40 ppm) or no nitrite was free of nitrosamines; 3) irradiated bacon with a commercial level of nitrite (120 ppm) contained only one third of the nitrosodimethylamine (NDMA) and nitrosopyrrolidine (NPYR) in comparison to non-irradiated samples; and 4) irradiation (30 kGy) destroyed more than 95 % of added NDMA and over 85 % of added NPYR in bacon. This indicated that irradiation has a destructive effect on preformed nitrosamines; an observation of great public health interest.

2.5. General remarks

In conclusion it can be said that, because of natural radiation resistance of some microorganisms, irradiation at low doses, in spite of its usefulness, cannot solve by itself all the problems related to the microbiological safety of foods. Solution of some of these problems usually requires appropriate combination treatment, e.g. irradiation with heat, irradiation with chemicals (nitrate and other salts), or appropriate storage conditions after irradiation, including proper temperature and packing. However, it should not be overlooked that not only does irradiation create another barrier to transmission of pathogenic organisms through food, especially the gram-negative organisms, but the survivors of irradiation are usually more sensitive to heat, drying, and other technological treatments of food. Any problems due to suppression of spoilage organisms by means of radiation processing at low doses is not likely to be greater than those encountered with other methods of partial preservation, e.g. pasteurization, salting and vacuum packing.

3. Enhanced pathogenicity and toxin formation

3.1. Enhanced pathogenicity

There is some evidence that the pathogenicity of infectious organisms is diminished by irradiation. Moreover, should irradiation-induced enhanced infectivity be a problem, this would have become apparent from the many wholesomeness studies on irradiated foods carried out so far (see Section VIII). Moreover, there is no evidence of undesirable effects arising from the irradiation of medical products or as a result of food irradiation which has already taken place in some countries, e.g. Japan, though this was relatively limited in amount.

Thousands of tons of feed for experimental animals are irradiated every year, being subject either to radappertization, radicidation, or radurization doses. No problems have so far been recognized in the animal kingdom from enhanced pathogenicity of surviving microbes.

Another consideration has been that radicidation and radurization might change the microbial community structure of fresh foods so that pathogens are able to multiply to dangerous levels before the normal association flora develops and metabolises sufficiently to spoil the food. There is no evidence to show that this risk is any greater than that found in foods treated by e.g. heat, where the same flora shift occurs.

3.2. Toxin formation by bacteria

As far as the effect of irradiation on toxin formation by bacteria is concerned, it was shown for Clostridium perfringens that the production of enterotoxin is not affected by low-dose irradiation treatment.

3.3. Toxin formation by fungi

Laboratory experiments have shown that aflatoxin production by mould (Aspergillus sp.) may either be increased (particularly if heavy inocula are incubated in irradiated autoclaved moistened foods), decreased, or unchanged in comparison with the parent strain.

Reported increases in mycotoxin production under some laboratory conditions are not relevant when assessing the microbiological safety of the irradiation process for three reasons : i) the increase in mycotoxin production per microorganism is more than balanced by the decrease in mycotoxin-producing organisms; ii) an increase in mycotoxin production per organism may be caused by the reduction in concentration of producing organisms and can also be induced by reducing the inoculum size; and iii) the potential mycotoxin hazard has not been enhanced by irradiation under practical conditions complying with the standards of the appropriate hygienic manufacturing practice.

In mycotoxin studies carried out under conditions more like those used in practice, increased formation of mycotoxins has not been found.

In studies on the toxin formation of Aspergillus flavus in lemon, tomato, apple, banana, carrot and grape species, no increase in toxin formation was noted, although repeated irradiation-growth cycles were carried out. In most instances the toxin formation decreased. Penicillium patulum also showed a decrease in toxin production with increasing doses. Irradiation with 2 kGy did not eliminate the growth of mycelia but inhibited the toxin production.

3.4. Increased antibiotic resistance

There is no evidence available to indicate that low-dose irradiation treatment would significantly increase the antibiotic resistance of bacteria.

4. Changes of taxonomically relevant characteristics

Several changes have been reported in irradiated microorganisms. They include changes in shape and size of cells, reduced vitality, and increased sensitivity to salt or other selective factors in the recovery medium as well as to lowered water activity. However, low-dose irradiation does not appear to change significantly the taxonomically relevant characteristics of the treated microorganisms.

In the most extensively investigated instance, that of Salmonella, general identity was not made doubtful. Though some reactions were weaker, serological typing remained possible even with recycled cells.

Moreover, a single irradiation treatment normally induces only transient changes in the surviving cells which revert after a few subcultures. This may well not be the case with repeated irradiation at high doses. Although, in general, the use of current methods for evaluating radiation-damaged microorganisms is appropriate, in some cases these methods should be specifically evaluated for their suitability to isolate radiation-damaged cells.

The same principle and methods used for the detection of sublethally injured bacteria following application of methods of preservation such as heat treatment, curing, etc., will apply to the detection of radiation-damaged cells. Such cells can be restored by a short

period of resuscitation on a favourable medium, after which their properties are normal. In food microbiology methods to detect stressed bacteria are already widely applied.

In 1977 the possible public health problem of modification of key characteristics of bacteria after irradiation has been most carefully reviewed by Ingram and Farkas who could not substantiate such claims. The findings of these workers were later confirmed by others.

5. Enhanced radiation resistance

Repeated exposure of survivors to sub-lethal radiations has often been shown in the laboratory to select populations with enhanced resistance to radiation, but there is no evidence that this can occur in actual practice. Indeed, similar increased resistance to other factors, e.g. heat, has been induced by comparable methods in the laboratory. Exposure to sunlight and ultraviolet irradiation can also cause mutations in microbes.

It has been possible under experimental conditions by repeated heat treatment of vegetative bacteria as well as spores and by subsequent 14 cyclic treatments of the most heat resistant strains, to increase the heat resistance of microorganism. This has, however, never been shown under practical conditions to give rise to the appearance of strains of bacteria with increased heat resistance in the environment or in food. Furthermore, if the strain having acquired a higher heat resistance is not kept continuously under constant pressure of heat, reversion to the normal heat resistance will occur after a few cycles without the selective pressure of heat. The same will apply under practical conditions apply to bacteria subjected to irradiation.

In an extreme example 84-cycle treatments increased the resistance of Salmonella to the level of Micrococcus radiodurans. This would imply that a dose of about 5 kGy is wholly ineffective. The significance of the possibilities for modifying key characteristics or acquired resistance clearly depends on the likelihood of repeated cyclic irradiation under favourable conditions. Most important among these conditions is that following each irradiation, there should be an opportunity for sufficient multiplication to restore the high degree of inactivation before the next irradiation. Recycling experiments have naturally been made under optimal conditions from this point of view, but such conditions are very unlikely to occur in practice. Moreover, if such regrowth did occur, it could be prevented by application of hygienic measures and/or temperature control.

In this context it should be pointed out that mutant strains tend to have weaker growth potential and diminished virulence, and the minimum growth temperature seems very likely to be raised rather than lowered by irradiation.

As mentioned under 2.1 it is also relevant to note in the context of radiation resistance that thousands of tons of feed for experimental animals are irradiated every year, and no problems have been identified so far. On the contrary, potential risks may be reduced significantly by the identification of critical control points in the production chain and in the irradiation facilities.

VIII TOXICOLOGICAL ASPECTS

A very large number of in vivo and in vitro toxicological investigations are available on a variety of irradiated foods (Table 9). Moreover, several studies have been carried out on isolated food components and selected radiolytic products. This section deals with these data as well as with those concerning radiation-sterilised animal feeds and with some observations available on human beings with an impaired immune response who have been fed with radiation-sterilized foods.

1. Studies on radiolytic products

A few studies are available on radiolytic products. Some 26 radiolytic products, selected from among those identified and quantified in beef fat irradiated at 60 kGy, were fed to mice in a modified three-generation reproduction study. The compounds investigated were the straight-chain alkanes and the 1-alkenes from C_5 to C_{17} in the proportions found after irradiation. The yield of these 26 radiolytic products was about 22 mg 100 g^{-1} irradiated beef fat and the average human intake was estimated at 0.77 mg kg^{-1} b.w. day^{-1}. Groups of 15 male and 15 female mice were therefore fed all 26 radiolytic products at 5.5 %, 1.8 % or 0.55 % in the diet. Additional groups were fed various combinations of 9, 8, 3 and 2 radiolytic products at concentrations ranging from 0.76 % to 2.1 % in the diet. Controls and pair-fed controls were included.

Feeding the combined 26 radiolytic products decreased survival and reduced bodyweight gain of both sexes of F_3 pups at weaning. The number of small hepatic necrotic foci was increased in a dose related manner compared to controls. A similar increase was noted for 9 of the radiolytic products, which included the C_{13}, C_{14} and C_{17} 1-alkenes, when fed at a single dose level. At 1.8 % of the diet only the F_3 males showed decreased body weight at weaning due to feeding 26 radiolytic products. Haematocrit values showed inconsistent decreases. No urinalysis or clinical chemistry was performed. Histopathology was reported on 9 major organs and showed no adverse effects apart from the hepatic lesions previously described.

Feeding the combined three C_{13}, C_{14} and C_{17} 1-alkenes at the single level of 3.82 % in the diet produced severe reproductive toxicity as shown by infertility, increased mortality of pups and absence of litters in the second generation.

The oral acute and 3-week subacute toxicity of 9 radiolytic products from among 35 products identified in aqueous extracts of starch irradiated at 3 kGy was determined in rats. The same 9 products were also fed to rats for 6 months. The compounds were : formaldehyde, acetaldehyde, malonaldehyde, glycolaldehyde, glyceraldehyde, glyoxal, formic acid, methyl alcohol and hydrogen peroxide. The compounds were administered in the drinking water as an aqueous solution with the various compounds present in the proportions found in aqueous extracts of irradiated starch.

The oral LD_{50} was 0.7 g kg^{-1} b.w. When given to rats for 3 weeks in their drinking water at 0.015, 0.072, 0.3 and 0.63 g kg^{-1} b.w. there was reduced fluid consumption at the highest dose level. No haematological or clinico-chemical abnormalities were found. Histopathology of 7 organs of the group fed the top dose level and of the stomach of all other groups showed epithelial hyperplasia of the forestomach at the top dose only.

In a 6-months study on 4 groups of 15 male and 15 female rats given 0.3, 0.1 and 0.072 g kg^{-1} b.w. in their drinking water a reduced fluid intake was seen at the top dose. Food intake and growth were comparable to controls in all test groups. Haematology, urinalysis and clinical chemistry including serum protein electrophoresis showed no consistent abnormal findings. Histopathology of 19 organs showed no lesions specific to the administration of the radiolysis products.

2. Studies on irradiated foods and food components

From Table 10 it appears that a very large number of food products have been submitted so far to toxicity testing. These food products can be gathered into several categories as indicated in Table 9. The studies evaluated by the Committee for each irradiated food are listed in the Annex.

2.1. Studies on isolated food components

Solutions of glucose and other sugars yield upon irradiation products cytotoxic and mutagenic for mammalian and non-mammalian cells. On the other hand, anhydrous glucose irradiated up to 50 kGy failed to induce any mutagenic effects in Drosophila or dominant lethal mutations in mice. Mutagenicity studies carried out on irradiated solutions of sucrose, glucose and ribose showed that the irradiated sugar solutions were mutagenic when tested in vitro on S.typhimurium, but not mutagenic in a host-mediated assay with Salmonella in mice. In vitro mutagenicity of solutions of fructose, glucose, sucrose or maltose, i.e. the four main sugars of mango, has been compared with that of irradiated ribose in several strains of Salmonella. After irradiation with 10 to 25 kGy the five sugars were all mutagenic for the S. typhimurium strain TA 100 in oxygenated solutions using the pre-incubation procedure, whereas no effects were seen in the strains TA 1535, TA 1537, TA 1538 and TA 98. The mutagenic effects observed in the TA 100 strain were much less evident in the absence of oxygen.

In order to identify the mutagenic compounds formed upon irradiation, a number of possible radiolytic products of sugars, some of which synthesised ad hoc, have been tested in the Salmonella spot test. The only mutagenic compounds detected were glyoxal, D-erythrohexo-2,3-diulose (which is however unstable) and D-arabinohexo-2-ulose (glucosone). Glucosone was implicated as the main mutagenic agent responsible for the observed effects. Despite the above-mentioned findings obtained with isolated fruit sugars, neither Kent mango juice nor the supernatant of the pulp of irradiated whole fruit exhibited any mutagenicity after irradiation at 20 kGy. Actually, addition of the supernatant of irradiated mango pulp to glucosone caused a considerable decrease in the mutagenic activity of glucosone.

Irradiation of pineapple, citrus and apple juices at rather high doses led to an increase in the frequency of chromosome breaks in onion root cells. The degradation of D-glucose present in apple juice has been studied at a radiation dose of 10 kGy and the identification was attempted of glyoxal, malonaldehyde and other dialdehydes thought to be cytotoxic and mutagenic. However only a small amount of glucose was decomposed under the conditions adopted.

Irradiated solutions of 2-deoxy-D-ribose and D-ribose, the sugar moieties of DNA and RNA respectively, were found to be mutagenic for S. typhimurium TA 100 and TA 98. Solutions of nucleic acid bases and nucleosides, saturated with either N_2, N_2O or O_2, were irradiated at 10 kGy and tested for mutagenicity for S. typhimurium with or without pre-incubation. Irradiated solutions of the nucleic acid bases were all non-mutagenic, while nucleosides were mutagenic for TA 100 in pre-incubation assays. Generally the mutagenic activity followed the order N_2O, N_2, O_2. The post-irradiation addition of catalase or of pH adjustment control did not affect the mutagenic response. On the whole these data indicate that the sugar moiety is the main substrate for the formation of mutagenic radiolytic products. The mutagenic activity was dependent on the quantity of carbonyl compounds produced and could be reduced or removed entirely by heating depending on the temperature used.

On the whole the above reported experiments indicate that the mutagenic products are only formed in fresh solutions of pure sugars following irradiation at high doses. Moreover these mutagenic products are converted to non-mutagenic substances upon heating and are not active *in vivo* possibly because of biotransformation to non-genotoxic substances.

2.2. Fruits

Very extensive and comprehensive data are available on 4 different fruits (mangoes, dates, strawberries, papayas). These data show clearly that mangoes, dates and papayas, irradiated up to 1 kGy, as well as strawberries irradiated up to 3 kGy can be incorporated in the diet of laboratory animals for their lifetime in large amounts without inducing any adverse health effects. Although not as complete as for the above-mentioned 4 fruits, a considerable amount of data including long-term and reproduction tests are available on mandarin oranges irradiated at 1.5 kGy, apples irradiated at 3 kGy, and prune-plums irradiated at 2 kGy. Moreover, limited *in vitro* and *in vivo* short-term testing has been carried out on bananas (0.3 kGy), oranges (1.5 kGy), apricots (2.5 kGy), and peaches (2.2 kGy). None of these studies showed any adverse results.

2.3. Vegetables

Onion and mushroom underwent extensive toxicity testing, whereas lettuce, celery, carrot and cauliflower have been submitted to the *Salmonella* reversion test only.

Onions irradiated up to 0.15 kGy have been submitted to long-term investigations with laboratory rodents, to several feeding reproduction studies and to a series of *in vitro* and *in vivo* genotoxicity studies. Early studies carried out at high dietary levels on onion were difficult to interpret because of the interference of naturally occuring toxic constituents causing haemolysis and anaemia. A number of more recent short- and long-term studies including genotoxicity have shown no adverse effects when irradiated onions were incorporated at a 2% level in the diet of rats and mice.

As far as mushrooms irradiated up to 3 kGy are concerned, the reproduction and teratogenicity studies did not reveal reasons for concern, but the long-term study carried out in the rat was not adequate and mutagenicity data are missing. Moreover, several adverse effects both with irradiated and non-irradiated mushrooms which are most likely due to naturally occurring toxic substances were observed in short-term investigations carried out with the rat and the dog at high dietary levels.

2.4. Cereals

Three irradiated cereals (i.e. wheat, rice and maize) have been submitted to extensive toxicological trials.

There are a large number of short-term, long-term, teratogenicity and *in vitro* and *in vivo* mutagenicity studies which did not show any health effects in test animals as a consequence of eating wheat irradiated up to 1 kGy and stored after irradiation.

Observations on children and feeding tests carried out with rats, mice and monkeys, have shown a slightly increased incidence of polyploidy in bone marrow cells or peripheral lymphocytes upon administration for several weeks of freshly irradiated (up to 1 kGy) wheat, but not in wheat *stored* for about three months after irradiation.

However, these effects were not confirmed by other authors. For instance in an experiment with rats, no increased polyploidy was detected after administration of wheat after 24 hours and 2 weeks after irradiation at 0.75 kGy. Similar results were obtained in another experiment carried out with rats fed wheat irradiated at 0.75 kGy after 2,4 and 8 weeks following irradiation. A long-term and reproduction experiment in the mouse using 50% in

the diet of fresh wheat irradiated at a very high dose (50 kGy) showed a significantly higher chromosomal damage in the sperm cells and reduced survival probability for the offspring of treated animals. These results which might be interpreted as a mutagenic effect, cannot be compared with those of the other above-mentioned investigations in view of the much higher irradiation dose used. Lastly, a reduced immune response was observed in rats administered for 15 weeks a feed containing 70 % freshly irradiated (at 0.75 kGy) wheat, but not in those treated with the same wheat 12 weeks after irradiation. It was noted that the animals, treated with freshly irradiated wheat, although exhibiting a reduced response, were still able to resist infections.

Long-term feeding studies with irradiated rice are available in the rat, mouse and dog. Multigeneration studies and genotoxicity studies have also been carried out. On the whole, these studies show that no adverse effects are associated with the long-term administration to several animal species of rice irradiated up to 1 kGy.

Maize has been much less studied than wheat and rice. Actually only one 3-generation reproduction study with mice is available.

2.5. Pulses

No long-term study is available for any pulse. Short-term studies indicated a reduced growth rate upon administration in the diet containing high levels of both irradiated and non-irradiated beans. Several mutagenicity studies with irradiated (1 kGy) dry beans did not show any adverse effects.

2.6. Spices and condiments

Only the Salmonella reversion test has been carried out on garlic powder irradiated up to 10 kGy, whereas several in vivo and in vitro mutagenicity data are available for onion powder irradiated up to 15 kGy. These data do not indicate any mutagenic potential for these irradiated seasonings. More extensive data have been produced for mild paprika, black pepper and a mixture of spices consisting of 55 % paprika, 14 % black pepper, 9 % allspice, 9 % coriander, 7 % majoram, 4 % caraway and 2 % nutmeg. When tested at dietary levels grossly exceeding possible intakes by human beings, paprika and pepper irradiated at 15 kGy did not induce any toxic effects in sub-chronic and teratogenic experiments with rats and in a series of mutagenicity investigations with mammals and bacteria. The same applies to the irradiated mixture of spices; only reduced food intake and body weight and increased liver weight were observed in rats treated for about 5 months with high dietary levels of both the irradiated and non-irradiated mixture of spices.

2.7. Miscellaneous plant foods

Miscellaneous plant foods include potatoes, cocoa beans and walnuts. Extensive long-term and reproduction studies in two rodent species have shown that the inclusion of cooked irradiated (0.15 kGy) potatoes in the diet does not induce any adverse effects. Moreover, several genotoxicity tests carried out with cooked potatoes or potato extracts confirmed the absence of mutagenic potential in irradiated potatoes.

Increased dominant lethal mutations were observed in mice following oral administration of an alcoholic extract of freshly irradiated (0.1 kGy) raw potatoes, but not in mice given a similar extract from non-irradiated raw potatoes or from irradiated potatoes stored for several weeks and steam-boiled. Moreover, the frequency of cells with chromosomal aberrations in the bone marrow of mice fed an extract from freshly irradiated potatoes was higher than that of animal fed extracts from stored and/or cooked irradiated potatoes. However, these studies were not confirmed in subsequent experiments. In fact, a dominant lethal study in mice, carried out in mice fed alcoholic extracts from freshly irradiated (0.12 kGy) potatoes, showed no effects on male fertility, pre-implantation loss

of ovulated ova and the total number of implantation sites. The micronucleus test was also used to study possible mutagenic effects of extracts of irradiated potatoes (0.1 kGy) obtained immediately following irradiation or after 24-h storage following irradiation. The results showed no significant differences between control and test animals.

Both irradiated (up to 5 kGy) and non-irradiated cocoa beans depressed growth and reduced food intake of rats when incorporated at high levels in the diet. These effects as well as those observed on fetal development and survival in rats are likely to be related to the high theobromine contents of the diets. Available mutagenicity studies did not show any mutagenic potential in irradiated cocoa beans.

Walnuts, irradiated at 1 kGy, have only been submitted to a reproduction study by feeding to mice.

2.8. Fish and fish products

The possible formation of toxic substances has been tested in several fish species (Table 10). The bulk of the investigations has been carried out on cod, Norway haddock and mackerel irradiated up to 2 kGy, and fish paste irradiated at 4.5 kGy. The overall findings indicate no adverse effects on animal health. A mixture of cod and Norway haddock, irradiated at 1.75 kGy, was boiled and then incorporated at 45 % into the diet of mice and rats. Both species were subjected to subchronic and long-term tests, multigeneration reproduction experiments as will as to teratogenicity investigations. The only effect observed was an increase of serum alkaline phosphate in the rats upon subchronic and chronic administration. This effect, however, was not reproduced in another experiment with rats, and not observed in mice and dogs which had been fed the irradiated product for 1 year. Cod, irradiated at 2.5 or 6 kGy, was submitted to subchronic and reproduction studies in rats and to several *in vivo* and *in vitro* genotoxicity tests. No adverse effects were detected in spite of the high dietary levels administered.

Irradiated mackerel (up to 2 kGy) was not only submitted to a number of *in vivo* mutagenicity tests, but also to subchronic, multigeneration and long-term investigations. Only effects that could be attributed to the high fat and high protein content of the diet were observed, regardless of whether or not the mackerel had been irradiated.

Although not tested as thouroughly as the aforementioned three species, several other irradiated species of fish did not present any reason for concern.

Marinated herring fillets, irradiated at 4.6 kGy, fed to rats at a dietary level of 50 %, did not affect their reproductive ability. Moreover, water and alcohol extracts of herring fillets irradiated at 12 kGy yielded inconclusive results in the Salmonella reversion test whereas those irradiated at 3 and 6 kGy did not show any mutagenic potential. Flounder and plaice, both irradiated at 1.75 kGy and then cooked, were tested on rats at a dietary level of 40 % in subchronic and reproduction experiments that did not show any effect which could be attributed to irradiation. Similar results were obtained with some short-term tests and mutagenicity tests that are available for the remaining members of this food category.

2.9. Shell fish

Only few wholesomeness studies have been carried out on crustaceans.

Seven groups, each of 10 males and 10 females were fed in a different 90-day study on Wistar rats. The various groups were fed standard laboratory diet (control group) or diets containing levels of 2.8% or 28% of shrimps which were either non-irradiated or had been irradiated at 1.5 or 3 KGy. No adverse effects were noted on growth, food intake,

haematological parameters and sgpt. Organ weights were determined for 13 organs and showed an increased relative weight of the kidneys, liver and ovaries in those groups fed 28 % shrimp irrespective of the radiation treatment. Histopathological examination of some 23 tissues showed fatty vacuolation of the liver as the only abnormality in all groups fed shrimp in their diet irrespective of irradiation. This effect was more noticeable at the 28 % dose level. No adverse toxicological effects could be ascribed to the feeding of irradiated shrimp for 3 months.

In a multigeneration study extending over 4 filial generations in Wistar rats two groups, each of 24 male and 20 female animals, were fed 25 % of either non-irradiated shrimp or shrimp irradiated at 2.5 kGy. All generations were observed for survival for 18 months. The fresh peeled shrimp were blanched at 80°C for 5 minutes, dehydrated at 55-60°C to 40 % moisture and irradiated at ambient temperature. The cooked shrimp were incorporated into the diet. Sixteen females and 8 males were used for the mating step. Growth and food efficiency were measured for 8 weeks in each generation. No differences due to radiation treatment were noted. Fertility, litter parameters and the weight gain of pups during lactation were comparable for both groups, the pups fed irradiated shrimp showing slightly higher weight gain. At 3 months of age 8 males and 8 females in each generation were sacrificed. The weights of 7 organs were comparable between the groups. Protein determinations on the liver were also found to be comparable as well as enzyme levels and haematology. The histopathology of major organs showed no abnormalities associated with the feeding of irradiated shrimp.

Four groups of 4 male and 4 female beagle dogs were fed for 2 years either a normal laboratory feed or a diet containing 50 % clam. The soft shell clams were irradiated either at 4 or 8 kGy and stored at 0° C for 30 days. The cooked clams were incorporated into the diet. No adverse effects were noted on growth, food efficienty, haematological parameters, reproductive function, litter size, birthanomalies, organ weights and histopathology of major organs. The weaning weight of all pups on the clam diet were slightly greater than those on normal laboratory chow.

None of the available studies have revealed any adverse toxicological effects due to the feeding of irradiated shell fish.

2.10. Meats

Cooked chicken irradiated up to 7 kGy has been submitted to two long-term studies in two rodent species, a three-generation reproduction study, some subchronic studies in the dog and in rodents, and several mutagenicity experiments in vivo and in vitro. On the whole, these data do not indicate any health problems resulting from the investigation of irradiated chicken. A number of toxicity investigations have also been carried out on chicken irradiated at much higher doses (up to 59 kGy). Two long-term studies in rats incorporating a multigeneration reproduction phase, used either 35 % of fresh boned chicken irradiated at 28 and 56 kGy and green beans irradiated similarly or chicken stew and cabbage irradiated at similar doses. The irradiated food were stored for 3-6 months before incorporation in the diet. No adverse effects were detected in these studies apart from some transient changes of enzyme levels of the intestinal mucosa only observed in the F_1 offspring. Beagle dogs were fed 35 % irradiated (28 or 56 kGy) chicken stew stored at room temperature for 3-6 months without adverse effects.

One feeding study in mice using chicken meat sterilised by irradiation at a dose of 59 kGy was reported to show a statistically significant increase in testicular tumours in the animals fed the irradiated food. Although the irradiation dose is much higher than those considered in the rest of this report the study may be considered relevant to the safety of food irradiated at lower doses since many of the radiolytic products present in chicken irradiated at an overall average dose of 59 kGy would also be present, though at lower

concentrations, in chicken irradiated at 7-10 kGy. It is conceivable that use of a higher dose would amplify any effects of irradiation, and this study might be a sensitive indication of a carcinogenic effect which could also be present at lower doses.

The study used CD-1 mice and employed five experimental groups. One group was fed a standard laboratory diet, the other four groups were fed laboratory diet plus chicken meat processed in one of the following four ways : frozen, heat sterilised, gamma-ray irradiated (59 kGy), electron irradiated (59 kGy). Two features of the study design were unconventional and both led to weaknesses in the study and problems of interpretation. Firstly, 40 animals of each sex were removed from the study at 15 weeks to act as breeder animals in a reproductive toxicology study being performed concurrently. These animals were returned to the study at 35 weeks but this caused problems in interpretation because the animals in each treatment group were no longer homogeneous. Furthermore, assignment of animals to the reproductive study was not made at random because low body weight animals in each group were excluded. The second unusual feature is that the test material (irradiated food) was only supplied at one dose level. The available evidence suggests that the two types of irradiated food tested (Co^{60} and electron irradiated) would have very similar, though not identical, types and levels of radiolytic products so that in effect the study was testing two similar processes at the same dose level. The lack of multiple dose levels makes it more difficult to determine the toxicological significance of any unusual findings since there is no dose-response information.

All the groups fed irradiated chicken had a higher calorie intake, more rapid weight gain and poorer survival than the group fed standard diet. Apart from these there were also some differences in tumour incidences between groups. These differences were statistically significant only for mammary gland tumours, where there was a decreased incidence in one of the irradiated chicken fed groups, and testicular tumours, where there was an increased incidence in both groups fed irradiated chicken.

For interstitial cell tumours of the testes the incidences (no. of animals with tumour/total number of animals) in each group were as follows :

Standard diet :	0/107
Frozen chicken :	1/162
Heat sterilised chicken :	0/111
gamma-ray irradiated chicken :	4/109
Electron irradiated chicken :	4/107

Statistical analyses were performed on the basis that the tumours were non-incidental, and gave p values below 0.05 for the following comparisons.

Frozen chicken vs. gamma-ray irradiated	: $p = 0.03$
Frozen chicken vs. electron irradiated	: $p = 0.03$
Frozen chicken vs. combined gamma-ray and electron irradiated	: $p = 0.02$

Because the p values for all three comparisons were below the value routinely required for statistical significance it was concluded that there was a significantly increased incidence of testicular tumours associated with consumption of irradiated chicken. All the testicular slides from this study were reviewed by the United States FDA's Center for Food Safety and Applied Nutrition (Division of Pathology). The main difference between the FDA and the study authors was that tumours considered to be derived from the same cell or origin were observed by FDA in the testes of mice in the non-irradiated group. These, together with interstitial cell tumours were all considered to belong to a classification of gonadal stromal tumour, and were analysed collectively. On this basis the following incidences were recorded.

Standard diet :	1/105	
Frozen chicken :	2/159	
Heat sterilised chicken :	1/109	
gamma-ray irradiated chicken :		3/107
Electron irradiated chicken :	4/106	

A statistical analysis was performed on the basis that these were non-lethal tumours and therefore an incidental (prevalence) analysis was appropriate. On this basis pairwise comparison failed to yield p values less than 0.05 except when all the groups not fed irradiated chicken were compared with both irradiated chicken groups. However, because of the large dietary differences between the group fed standard diet and the chicken fed control groups, pooling of results for the three control groups was not considered valid. In addition to the statistical results, other factors were noted which indicated that the testicular tumours were not related to the consumption of irradiated food; in particular the lack of an increase in interstitial cell hyperplasia and lack of evidence of progression from hyperplasia to neoplasia, the lack of other lesions in the testes indicative of a toxic effect of irradiated chicken, and the fact that all the testicular tumours were unilateral. As a result of all these considerations the FDA concluded that the study failed to provide evidence of a carcinogenic response as a result of consumption of irradiated chicken meat.

The data on testicular tumours was also reviewed independently by the United States National Toxicology Programme, who had similar criticisms of the study report. They concluded that the study could not be characterized as demonstrating a carcinogenic response to consumption of irradiated chicken meat.

Taking into account the study report and the two independent reviews we are satisfied that an appropriate histopathological classification and statistical analysis applied to this study does not show any carcinogenic effect of consumption of irradiation - sterilised chicken meat, and that this study has no unfavourable implications regarding the safety and wholesomeness of poultry irradiated at the doses recommended in our report.

3. Human experience

In a number of countries irradiation at doses of 25 kGy or more has been used over many years to achieve effective sterilization of the diets of patients suffering from diseases or undergoing treatments which make them particularly susceptible to infection. It would be inappropriate to draw general conclusions about the nutritional and toxicological status of irradiated food from this application since people with special nutritional requirements and clinical problems are involved. However, no specific adverse nutritional or toxicological effects have been reported following the use of these diets, and this observation indicates that high doses of radiation do not have major effects on the nutritional content or toxicological properties of food. Irradiated food has also been used in the preparation of other specialized diets, in particular by astronauts both from the USA and the USSR. Although detailed nutritional studies have not been reported on astronauts, the consumption of irradiated food did not cause any overt adverse nutritional or toxicological effects in this closely monitored group.

Certain irradiated foods have been consumed in some countries for more than 20 years.

4. Studies on irradiated feeds

There is a considerable amount of toxicological data relating to the use of irradiated laboratory and commercial diets. A radiation dose of 15 kGy appeared active in preventing spoilage of commercial diets, but some vegetative organisms are not effectively removed at

irradiation doses of less than 24 kGy. Moreover, to kill viruses doses in excess of 40 kGy are required. It appears that irradiation of animal feeds takes place generally at doses considerably higher than most human food products.

4.1. Laboratory animal diets

Reproduction over several successive generations of mice kept on diets irradiated up to 25 kGy did not appear to be different from those fed on autoclaved or ethylene oxide-fumigated diets. Moreover, mice maintained on the irradiated diet exhibited greater weight gain than those fed the autoclaved diet, but similar weight gain to those fed the fumigated diet. Similar results are also vailable for the rat. In four multigeneration feeding studies in rats, kept on nutritionally supplemented test diets irradiated up to 60 kGy, no adverse effects were observed in respect of growth, haematology, reproduction and tumour incidence in the parent or successive generations. Two comparative studies are available in SPF Wistar rats placed on amino acid and vitamin-supplemented feeds sterilized either by irradiation at 50 kGy or by autoclaving at 110°C or at 120°C. Rats were maintained on one of these three diets for two weeks prior to mating. No differences were observed among the fertility indices of the three groups. Litter size, growth rate, feed consumption and general health were monitored in the resulting offsprings of several generations obtained during 18 months without detecting any significant difference among the three diets, apart from some differences in urine and blood biochemistry of the animals of the F_1 generation that underwent a 90-day feeding study.

A 15-20 % reduction in the lymphocyte count was seen in male Sprague-Dawley rats kept on freshly-irradiated or stored-irradiated (at or above 6 kGy) standard laboratory diets, with older rats being more susceptible to this effect. Moreover, preliminary data from an experiment in which an irradiated diet (2-200 kGy) was fed for one month indicated an increase of absolute and relative weight of the thymus, but not of the spleen. The agent responsible for the lymphocytopaenia induced by irradiated laboratory diets has not been identified nor the action mechanisms clarified. Studies conducted for the International Project at Karlsruhe failed to confirm an effect of feeding irradiated diets on number of lymphocytes in the peripheral circulation. However, a possible effect on the immune system has still not been ruled out, particularly in the light of the Russian study indicating that long-term feeding of irradiated diet at 0.5, 5 or 56 kGy induced a dose-dependent kidney damage possibly mediated through an immunological mechanism.

Animal laboratory diets have been shown to be mutagenic when tested immediately after irradiation at 30 kGy in Salmonella strain TA 1530 and strain G 46 in a host-mediated assay involving a large number of mice. Ethanol and water extracts, but not ether extracts, of these diets were also mutagenic in this system. The effect of reducing the irradiation dose used (i.e. 7.5 kGy) still produced an increased response in terms of the incidence of histidine revertants in one experiment with Salmonella TA 1530. The mutagens in irradiated feeds are probably relatively short lived. This was indicated by another study with essentially the same test system that showed a slight increase in the level of back mutations upon treatment with a diet immediately after irradiation at 30 kGy, whereas no mutagenic activity at all could be detected if the irradiated pellets were stored for two weeks before administration to mice. Several dominant lethal assays have been carried out with diets irradiated at 25 or 45 kGy with no evidence of mutagenicity. An experiment with animal feed containing wheat revealed a slightly increased incidence of polyploidy associated with freshly irradiated feed at doses over 20 kGy, and no effect at doses below 20 kGy, or when feed irradiated at high doses (up to 45 kGy) was used after storage for 6 weeks.

In a series of experiments on rats the effect of irradiated semi-synthetic feed (at 10 kGy) has been compared with the effect of heat-treated semi-synthetic feed (100°C for 60 minutes). The feed was stored for at least 8 days at 4°C before being used. The rats were fed for 9 weeks and were immunised once weekly in each of the last three weeks with the

antigens tetanus toxin and red blood cells from sheep. Blood samples were then taken and examined for their content of antibodies for the antigens in question. In a total of four experiments, irradiated feed did not affect the immune response, although heat-treated feed in one of the experiments produced an improved response compared to non-irradiated feed.

4.2. Commercial feedstuffs

Whole cereals, irradiated at 0.2 kGy, were fed for 6 months at 75 % of the diet to battery hens; no significant effects were detected on body weight gain, egg production, total weight of eggs and mean egg weight. Post-mortem examination revealed no evidence of any adverse effects due to the irradiated diet. Similar data are also available for whole diet irradiated at 10 kGy. A three-generation study was carried out with chickens administered diets irradiated at 10 kGy or 35 kGy. No effects were detected on growth response, feed efficiency, and thiamine and riboflavine content of muscle and liver. One report is available indicating a decrease in egg production during a multigeneration study on chickens fed a diet irradiated at 30 kGy, but this effect was attributed to partial destruction of vitamins E and D.

A considerable amount of data is available on pigs fed for 3-4 months vitamin-supplemented diets irradiated at doses up to 20 kGy. No effects of the irradiation were detected on growth rate, feed intake, and blood tests; some non-specific histological changes were observed in the pigs fed some irradiated diets. The effects on pig reproduction of vitamin-supplemented feed sterilized by irradiation at 50 kGy have been compared with those of feed sterilized by heat treatment (10 minutes at 120°C) and of untreated feed. Parameters monitored included for the parent, F_1 and F_2 generation, fertility index, average duration of pregnancy, piglet weight at birth, size and weight of litters, viability index, lactation index, growth rate of piglets and general state of health. In no case did the animals treated with irradiated feed performed less well than controls, whereas less satisfactory results were reported for the pigs fed the heat-sterilized diet. Similar results were obtained in a 4-months fattening study on F_1 animals. A long-term pig study suggested that irradiation of the feed may induce haematological changes; in fact an increased number of neutrophile leucocytes was observed in pigs given the irradiated diet from the age of 11 months for 23 months.

5. Special toxicological considerations

5.1. Early mutagenicity data

As early as 1969 a number of both positive and negative results was available from various types of mutagenicity experiments on irradiated foods, most of which had been performed *in vitro*. These results bear the mark of the early stage of development of these methods and are consequently of less relevance to an evaluation made today.

This section deals with these early mutagenicity data, whereas the more recent data produced in order to assess whether the effects identified in these early studies could manifest themselves in mammals exposed to irradiated foods and food components have already been discussed in the previous subsections of Section VIII.

In 1969 the Joint Expert Committee for Food Irradiation (JECFI) had available the results from various experiments with irradiated culture media or media additives. Experiments with *Drosophila*, amongst others, had revealed an increased mutation frequency following exposure to irradiated media, which was considered to indicate that mutagenic substances could be produced by irradiation. A second experiment with *Drosophila* revealed no mutagenic effects, however.

Besides these experiments, a number of other in vitro experiments did report the findings of mutagenic and other toxic effects of various kinds of irradiated media or media additives.

An early in vitro experiment demonstrated that the growth of E. coli bacteria is restricted more by the addition of potato extracts from irradiated potatoes than from non-irradiated potatoes. An increase in the incidence of micronuclei was reported in cells of barley cultured in a growth medium to which irradiated mashed potatoes had been added, and it was shown that the cell division in vitro of carrot cells stimulated with coconut milk could be inhibited if the coconut milk was irradiated beforehand. In addition to this an irradiated medium was also shown to have a strongly inhibiting effect on the growth of the carrot cells. It was also demonstrated that irradiated sugar could bring about abnormal chromosome divisions in plant cells taken from Tradescantia paludosa. The same author found that the irradiation of the culture media produced a fall in pH from 7.0 to 3.3.

The experiments referred to above and a series of corresponding earlier experiments were evaluated in 1969. It was stated then that these positive effects could be related in the majority of cases to a number of comparatively simple chemical and biochemical factors which may be assumed to be of minor significance in healthy mammals.

Firstly, the irradiation process causes hydrogen peroxide and other peroxidised components to be produced in the irradiated material. It was found from similar experiments that the cytotoxic and mutagenic effects were suppressed if the enzyme catalase was added. Catalase, which is found in healthy mammals, has the ability to transform peroxy compounds, and since it is known that peroxy compounds can be mutagenic and cytotoxic, it may be assumed that a large proportion of the effects observed are due to the presence of peroxy compounds produced by the irradiation process. It was shown, amongst other things, that a mutagenic effect can be produced only in E. coli strains which had no catalase enzyme activity, whereas E. coli strains with catalase enzyme activity are not responsive. It was also found that the relationship between the cell density and the concentration of the irradiated product in the medium is critical. The effects are not produced at high cell densities since the metabolic activity of the cells is then able to eliminate the radiolytic products in the medium.

Secondly, the irradiation of media also brings about a steep fall in the pH, which may be taken as an adequate explanation for a number of the effects identified.

5.2. Irradiation of polyunsaturated fats

When certain foods become rancid on being left to stand, especially after having been heated, it can be related to the fact, that a number of changes takes place in the polyunsaturated fatty acids. The process which is due to the effect of free radicals in the presence of oxygen is peroxidation. This process results in the formation of, amongst other things, hydroperoxides and a number of carbonyl compounds, such as malonic aldehyde.

The process can also be initiated by the irradiation of foods containing unsaturated fats, especially under unfavourable conditions. As is already familiar from investigations of rancidity, the process is slowed down to a considerable degree if the food is irradiated under oxygen-free conditions (e.g. in a nitrogen atmosphere), at low temperature, and if antioxidants are present in the food.

Peroxidation is believed to be the cause of a number of toxic effects in biological systems. The tissue cells contain unsaturated fatty acids, in particular in those fats which are known as the phospholipids, which serve the function of building blocks in the membranes, for example of mitochondria, lysosomes and the endoplasmic reticulum. The peroxidative destruction of the unsaturated fatty acids in the membranes will take place under attack by free radicals and activated forms of oxygen. These reactions can be

initiated by a great many chemical substances. The result can be the disruption of the organised structure of the membranes, resulting in the loss of their specialised functions. Additional free radicals and peroxides, which could also attack other important substances in the cells, are formed during the peroxide chain reaction.

The effect of such attacks on the cells will depend on the degree of peroxidation, and ranges from the loss of a small number of specialised processes to cell death. In certain cases the development of cancer has been linked to the presence of free radicals and peroxidation processes; a specific suggestion has also been put forward to the effect that cancer resulting from exposure to ultraviolet light is attributable to the formation of cholesterol-alpha-oxide in the cell. These hypothesis are based in particular on the fact that the effects are often countered by antioxidants.

A series of toxic effects are reported in test animals following the direct injection of pure hydroperoxides of fatty acids. The most significant effects of various hydroperoxides in rats have been the massive diffusion of tissue fluid into the abdominal cavity (ascites) and cell death in particular in the liver and among the red blood cells. It has been demonstrated at the biochemical level that the hydroperoxides are able to inhibit the respiratory chain of the cells.

The effect is very much weaker, however, if these substances are administered perorally, apparently because the substances are destroyed to a very great extent in the gastro-intestinal tract. Only at very high dosages is a local toxic effect observed in the gastro-intestinal tract.

A number of carbonyl compounds is produced in the course of the peroxidation of unsaturated fatty acids. Such compounds can also be formed from carbohydrates, both by irradiation and by heating. Of these compounds, malonic aldehyde has been investigated to the greatest extent. Malonic aldehyde is mutagenic in the Salmonella reversion test and in various cell culture systems, and is reported to be capable of producing liver cancer in mice.

As has already been mentioned in the previous subsections of Section VIII, the many available experiments in no way indicate that the irradiation of the foods mentioned at the stated dosages will cause humans to be exposed to toxicologically harmful quantities of products formed through peroxidation of polyunsaturated fats. This section deals with the specific considerations related to the extent of the changes that the ingestion of peroxidised fat could produce and which are not usually revealed in conventional animal experiments and in in vitro experiments and with the special biochemical studies undertaken to test this possibility.

As unsaturated fatty acids form part of the structure of the endoplasmatic reticulum (EPR) membrane, a series of experiments has been conducted to investigate whether the daily administration of irradiated fats can change the ability of the test animals to metabolize xenobiotic substances, and thereby indirectly whether any changes have occurred in the EPR membrane.

Pork fat (5 % polyunsaturated) has been chosen as a typical saturated fat, whereas herring oil (80 % polyunsaturated) has been chosen as a typical unsaturated fat. The irradiation of herring oil in the range between 2 and 10 kGy resulted in an approximately linear increase in the formation of peroxides, unsaturated aldehydes and malonic aldehyde. The formation of these substances is inhibited to a considerable extent by the addition of antioxidants. In maize oil, which contains vitamin E as a natural antioxidant, these substances are formed only to a small extent during irradiation. No antioxidant has been added to the herring oil, which was used in the animal experiments.

Only very low mixed function oxidase (MOF) activity was observed in rats fed a fat-free diet.

Saturated fat added as pork fat (present in the feed at 10 %) produces an increased level of activity compared to the fat-free diet, although still at a low level. No change in MFO activity has been noted as a result of the irradiation of pork fat, or as a result of the irradiation of the carbohydrate and protein in the feed.

Unsaturated fat given as the herring oil (present at 10 % in the food) has caused high MFO activity. When the food contains 10 % irradiated (at 2.5 - 5 kGy) herring oil, the MFO activity is generally lower than with non-irradiated herring oil. This may be taken as an indication that irradiation produces changes in the unsaturated fatty acids in the herring oil. This is also demonstrated by analytical chemical determination, where it is shown that an irradiation dosage of 10 kGy causes the destruction of a number of polyunsaturated fatty acids, which in turn results in a change in the fatty acid composition in the EPR within 2-4 days.

In spite of the lower basal MFO activity, however, a slight increase in cytochromes P-450 and P-448 activity is observed after induction with phenobarbital and 3-methyl cholanthrene respectively in animals which had been given irradiated herring oil, by comparison with animals which had been given non-irradiated herring oil.

The measured changes in activity are small, in spite of the fact that the quantity of polyunsaturated fat administered has been high. The changes are considered to be without toxicological significance for the transformation of xenobiotic compounds in the human body.

TABLES

TABLE 1. EFFECTS AND POSSIBLE APPLICATIONS OF FOOD IRRADIATION

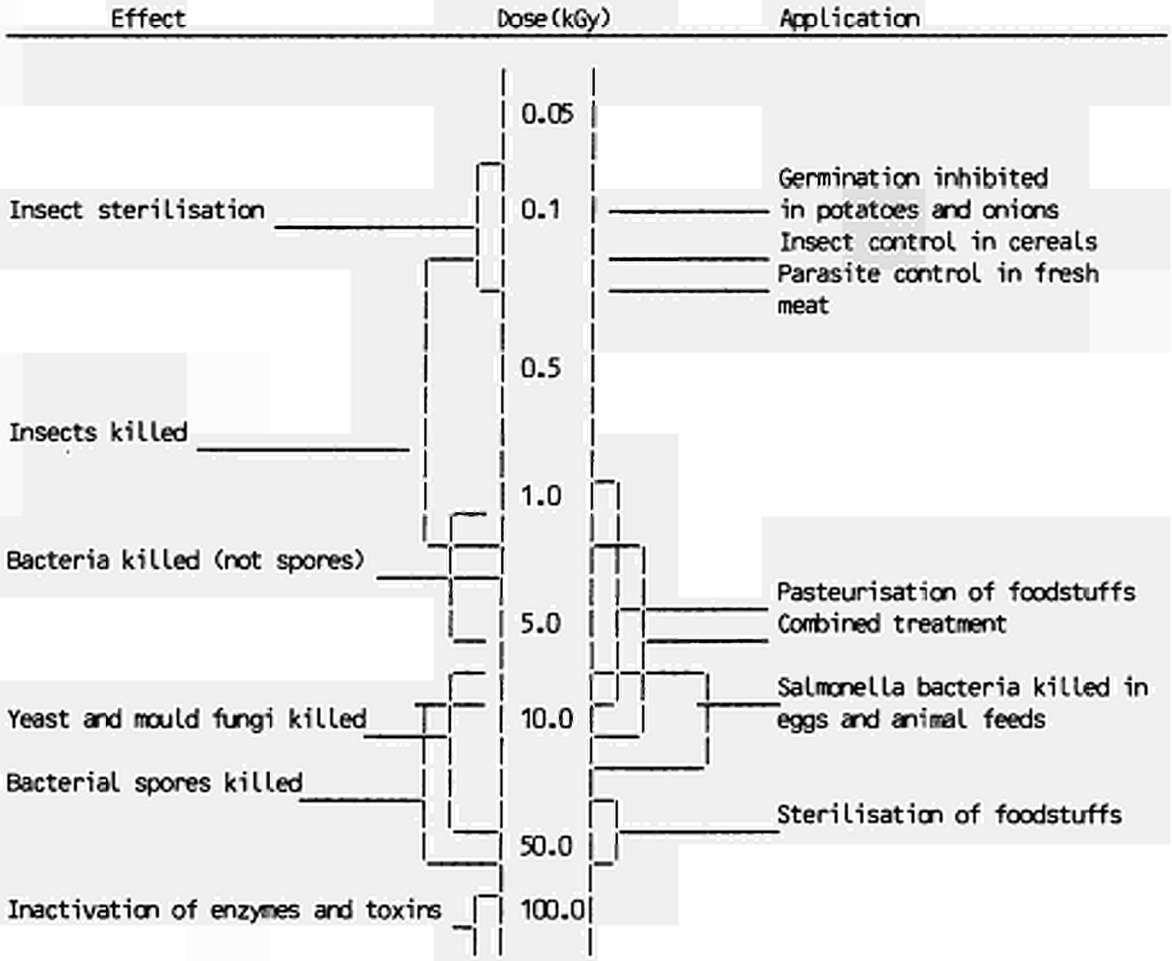

TABLE 2. HYDROCARBONS ISOLATED FROM PORK FAT, COCONUT BUTTER, OLIVE OIL AND SUNFLOWER OIL AFTER IRRADIATION AT 60 kGy (B) AND AFTER HEATING TO 170°C FOR 24 HOURS (H)

Hydrocarbons	Pork fat 1)		Coconut butter 2)		Olive oil 3)		Sunflower oil 4)	
	B	H	B	H	B	H	B	H
Octane		+						
n-Octane	+	+	+	+			+	+
n-Nonane	+	+	+	+			+	+
n-Decane	+	+	+	+	+	+	+	
Undecane				+	+	+		
Unclecane				+	+			
n-Undecane	+	+	+	+	+	+	+	
n-Dodecane	+	+	+	+	+	+	+	+
n-Tridecane	+	+	+	+	+	+	+	+
n-Tetradecane	+	+	+	+	+	+	+	+
n-Pentadecane	+	+	+	+	+	+	+	+
n-Hexadecane		+	+	+	+	+	+	+
n-Heptadecane							+	
n-Heptadecane	+	+	+	+			+	+
n-Octadecane	+	+	+	+	+	+		+
Octene-(1)			+				+	
Nonene-(1)			+	+		+		+
Decene-(1)	+	+	+	+	+	+		
Undecene-(1)			+	+	+			
Dodecene					+	+		+
Dedecene-(1)			+	+	+	+	+	
Tridecene					+		+	+
Tridecene-(1)	+	+	+	+	+	+	+	+
Tetradecene-(1)	+	+	+		+	+	+	+
Tetradecene					+	+	+	+
Pentadecene					+	+	+	+
Pentadecene-(1)	+	+	+	+	+	+	+	
Hexadecene		+						
Hexadecene-(1)	+	+	+	+	+	+	+	+
Heptadecene					+	+	+	
Heptadecene-(1)	+	+	+	+			+	+

TABLE 2. (continued)

Hydrocarbons	Pork fat 1) B	Pork fat 1) H	Coconut butter 2) B	Coconut butter 2) H	Olive oil 3) B	Olive oil 3) H	Sunflower oil 4) B	Sunflower oil 4) H
Octadecene					+	+		
Octadecene-(1)		+	+	+	+	+	+	+
Tetradecadiene	+				+		+	
Pentadecadiene	+				+		+	
Hexadecadiene	+		+		+		+	
Heptadecadiene	+				+		+	+
Heptadecadiene					+			
Heptadecadiene					+			
Hexadecatriene							+	
Heptadecatriene							+	
Butylcyclohexene		+		+		+		+
Pentylcyclohexene		+				+		
Hexylcyclohexene		+		+		+		
Heptylcyclohexene		+		+		+		+

1) Principal fatty acid:	Oleic acid:	Principal break-down product after irradiation: hexadecadiene Principal break-down product after heating: cyclic hydrocarbons
2) Principal fatty acid:	Lauric acid:	Principal break-down product after irradiation: undecane Principal break-down product after heating: undecane
3) Principal fatty acid	Linoleic acid:	Principal break-down product after irradiation: hexadecadiene Principal break-down product after heating: butyl cyclohexane
4) Principal fatty acid:	Oleic acid	Principal break-down product after irradiation: hexadecadiene Principal break-down product after heating: cyclic hydrocarbons

Taken from "Levnedsmiddelbestraling", the Danish Food Institute (1982)

TABLE 3. : ESTIMATES OF RADIOLYTIC PRODUCTS (RPs) IN IRRADIATED FOODS

Radiation Dose (krad)	G_T Events/100eV	(a)Yield of all RPs in Food (mmol/kg)	Yield of all RPs if MW=300 (mg/kg)	(b)Yield of URPs(c) (mg/kg)
10	1	0.01	3	0.3
50	1	0.05	15	1.5
100	1	0.10	30	3.0
1,000	1	1.0	300	30.0

(a) - Yield (in mmol/kg) = Dose (krad) x G_T x 10^{-3}

(b) - Assumes only 10% of RPs are unique (see text)

(c) - URPs = Unique radiolytic products

TABLE 4. MOST SUITABLE METHODS FOR IDENTIFICATION OF DIFFERENT IRRADIATED SPICES

more efficient results by TL	more efficient results by CL	very similar results by both methods	only CL investigated
Caraway (10)	Cardamom (3)	Basil (12)	Aniseed (12)
Chilli (12)	Celery (12)	Fennel, ground (6)	Cloves, whole flower-bud (6)
Chive (3)	Cinnamon (6)	Onion (6)	Coriander, single seed (8)
Cloves, ground (1)	Juniper berries, ground (8)	Pepper, white (3)	Fennel, single seed (8)
Cumin (8)	Shallot (2)	Mushrooms:	Juniper berries, whole berry (6)
Curcuma (8)		Champignon (3)	Pimento, whole berry (3)
Curry (8)		Chanterelle (3)	Sesame (7)
Garlic (3)		Morel (3)	
Coriander, ground (8)		Edible boletus (3)	
Paprika (12)		Ringed boletus (3)	
Parsley (6)			
Pepper, black (6)			
Pimento, ground (6)			
Sage (6)			
Tarragon (11)			

TL means Thermoluminescence, CL means Chemiluminescence.
( ) number of months after the radiation treatment, during which treatment identification is still possible (some of the investigations have been done only with samples from one manufacturer of a spice type).

TABLE 5. : NUTRITIONAL ASPECTS OF FOOD IRRADIATION : EXAMPLES OF CHANGES INDUCED IN FISH AND MEAT

Food item	Radiation Doses (kGy)	Chemical changes observed	Reference
1. Mackerel	1 - 45	Upon storage at -22°C in plastic bags, no changes in amino acids. Niacin was stable even at the highest dose, but 3kGy induced losses of 15 and 26% for thiamine and pyridoxine, respectively. At higher doses, thiamine was almost completely destroyed.	Underdal et al (1976)
	up to 10	No effect on oxidative deterioration	Ghadi et al (1978)
2. Haddock and codfish	2 - 56	Dose-dependent formation of hydrocarbons, carbonyl and sulphur compounds	Angelini et al 1975) Taub et al (1976)
3. Herring fillets and oil	up to 50	Irradiation under vacuum at 0°C did not cause any destruction of polyunsaturated fatty acids in fillets. 50% destruction of the polyunsaturated fatty acids was observed in oil.	Adam et al (1982) Murray (1980)
4. Shrimp	2 - 45	Stability of tryptophan was measured after storage at various temperature. Slight losses were observed.	Antunes and Novak(1978)
5. Beef	47 - 71	Irradiation at -40°C did not cause any destruction of cysteine, methionine and tryptophan both immediately after irradiation and on re-examination after 15 months storage.	Elias P.S. (1985) "Developments in meat science - 3". Ed. R. Lawrie; Elsevier Applied Science Publication Ltd, Essex, UK, pp 115-153
6. Pork	1	5% loss of vit.B1 immediately after irradiation at 0°C and an additional 38% loss occurred after 4 months storage at 0°C	Elias and Cohen (1977)
	6	-irradiation of fried pork under vacuum caused no loss of linoleic, linolenic and arachidonic acid also after 15 days of storage at room temperature.	

5 - 30	74-95% loss of vit. B
58	Less than 10% destruction of pantothenic acid and no destruction of folacin.

TABLE 6. NUTRITIONAL ASPECTS OF FOOD IRRADIATION : EXAMPLES OF CHANGES INDUCED IN FRUITS, VEGETABLES AND TUBERS

Food item	Radiation dose (kGy)	Chemical changes observed	Reference
1. Mangoes	up to 2	Slight losses in ascorbic acid and carotene. No changes in levels of riboflavin, niacin, thiamine, fat, protein, sugar and minerals.	Beyers et al (1979) Thomas and Beyers (1979) Beyers and Thomas (1979) Blakesley et al (1979)
2. Papaya	up to 1	Slight losses in ascorbic acid and carotene. No adverse effects on "volatile profile".	
3. Strawberry	up to 3	No changes in "volatile profile" nor in chemical constituents.	
4. Litchi		Slight losses in vitamin C and carotene	
5. Banana	0.2-0.4	No change	Loaharanu (1971)
6a. Orange	1 - 2	3 and 28% loss of vitamin C respectively	Josephson (1979)
6b. Orange juice	2 - 7.5	23 and 48% losses of vitamin C respectively. Increased peel browning at 2 kGy	Belli-Donini and Baraldi (1977) Keskin (1980)
7. Dates	up to 1.5	No change in free fatty acids and flavour	Jaddou & Al-Hakim (1978) Auda & Al-Wandawi (1980)
	up to 5	No change in reducing sugar content, major carbohydrate, and protein. No malonaldehyde detected	Auda et al (1978)
	up to 10	No change in amino-acid composition	
8. Tomato	1.5-3.0	9-14% loss of vitamin C, decreased sugar level; decreased resistance to penetration and increased decay	Magaudda et al (1978) Josephson (1979)
9. Carrot	0.8	No change in composition with regard to sugars, nitrogen, free amino acids and pectins. Slight decrease in vitamin C and -carotene	Ismail et al (1977) Baraldi et al (1979)
10 Potato	up to 0.15	Slight loss in vitamin C During after irradiation storage vitamin C disappeared more rapidly than in non-irradiated potatoes Unchanged thiamine and riboflavin Some changes in the concentration	Baraldi and Miuccio (1975) Bellomonte et al (1978) Azar et al (1980) Josephson (1979)

		of free amino acids, but not in amino acid make up of proteins.	
11. Cassava	0.62; 1.25; 2.5; 5	At 1.25 kGy and above, total protein was reduced by 1%; lysine, arginine and phenyl-alanine increased slightly, whereas soluble carbohydrates, hemicellulose and cellulose slightly decreased.	Ogbadu (1979)
12. Onion	up to 0.15	In the presence of air, some conversion of ascorbic acid to dehydroascorbic acid was observed. No change occurred in the amino acid composition.	Ghods et al (1966) Mahmoud et al (1978)
13. Endive	up to 1	Slight loss of vitamin C	Langerak (1978)

TABLE 7. NUTRITIONAL ASPECTS OF FOOD IRRADIATION : EXAMPLES OF CHANGES INDUCED IN CEREALS AND PULSES

Food item	Radiation dose (kGy)	Chemical changes observed	Reference
1. Rice	up to 1	No effect on nitrogen content, amino acid composition. Doses higher than 1 kGy reduce levels of thiamine, riboflavin, niacin, and pyridoxine.	Azar et al (1979)
2. Maize	0.25-3.0	After 4 years storage, protein quality and vitamin content were unaffected.	Chain et al (1977)
3. Wheat	0.15-1.0	No change in moisture, ash, nitrogen, protein, fat, carbohydrate and lysine content. Vitamin E and vitamin B complex did not show much change, except for thiamine which may be lost to some extent.	WHO (1977)
	2	20% loss of vitamin B_1 Slight loss of vitamin E	WHO (1977)
	10	No change in total lipids and in amounts and constituents of non-polar and polar lipids. However, there was a slight loss of unsaturated fatty acids and bound lipid. Free lipids increased. No change reported in amino acids.	Rao et al (1978)
4. Sorghum and Millet	0.2	No changes in contents of amino acids, vitamins B and B_2, niacin and panthothenic acid.	Adrian and Frayssinet (1975)
5. Oatmeal	0.25	23% loss of vitamin B_1 after 3 months of storage and 85% loss after 8 months. Non-irradiated oatmeal only showed 7% and 30% loss, respectively.	Diehl (1979)
	1	The loss of vitamin E after 8 months of storage could be reduced from 56% to 5% by packing in a nitrogen atmosphere.	
6. Kidney beans	0.15	Upon irradiation beans showed an early loss of riboflavin, increased hardening and development of an undesirable taste. After 5 months storage, irradiated and non-irradiated beans did not differ.	Fonseca et al (1979)

7. Sunflower	0.20	No changes in quality and composition of fatty acids of the oil.	El Zeani et al (1977)
8. Cocoa beans	up to 5	No changes in reducing sugars, total amino acids, total fat and protein of beans. No changes in chemical composition of fat.	WHO (1981)
9. Legume beans	1; 10	No radiation damage	Cohelo et al (1978)
10. Nuts	up to 1	20-30% loss of vitamin E	Elias and Cohen (1977)

TABLE 8. APPROXIMATE REDUCTION DOSES (kGy) TO REDUCE VIABLE NUMBERS OF VARIOUS MICRO-ORGANISMS ONE MILLIONFOLD

Gram-negative bacteria	
Escherichia coli	1.5 - 3.0
Salmonella spp.	3.0 - 5.0
Shigella spp.	1.5 - 2.0
Acinetobacter spp.	0.5 - 1.0
Pseudomonas spp.	0.5 - 2.0
Proteus	0.5 - 1.5
Vibrio parahaemolyticus	0.5 - 1.0
Moraxella	5.0 - 7.0
Gram-positive bacteria	
Bacillus vegetati	2 - 3
Bacillus cereus	20 - 30
Bacillus stearothermophilus	10 - 20
Clostridium botulinum A and B	10 - 30
Clostridium botulinum E	10 - 20
Clostridium perfringens	20 - 30
Leuconostoc	0.5 - 3.0
Lactobarillus	2.0 - 7.5
Micrococcus spp.	2 - 5
Micrococcus radiodurans	30
Micrococcus roseus	30
Staphylococcus aureus	0.5 - 5.0
Streptococcus	5.0 - 7.5
Streptococcus faecalis	10 - 20
Moulds and yeasts	
Aspergillus	1.5 - 5.0
Penicillium	0.5 - 2.0
Saccharomyces	5 - 10
Viruses	
Different species	30
Foot and mouth disease	10 - 30

TABLE 9. SOME IRRADIATED FOODS THAT HAVE BEEN SUBMITTED TO TOXICOLOGICAL INVESTIGATION

1. FRUITS
   1. Apples**
   2. Apricots*
   3. Bananas*
   4. Dates***
   5. Mandarin oranges**
   6. Mangoes***
   7. Oranges*
   8. Papayas***
   9. Peaches*
   10. Prune-plums**
   11. Strawberries***

2. VEGETABLES
   1. Carrot*
   2. Celery*
   3. Cauliflower*
   4. Lettuce*
   5. Mushroom**
   6. Onion***

3. CEREALS
   1. Maize*
   2. Rice***
   3. Wheat***

4. PULSES
   1. Beans**
   2. Lentils*
   3. Peas**

5. SPICES AND CONDIMENTS
   1. Garlic (powder)*
   2. Mixture of spices**
   3. Onion (powder)**
   4. Paprika**
   5. Pepper (black)**

6. MISCELLANEOUS PLANT FOODS
   1. Cocoa beans**
   2. Potatoes***
   3. Walnuts*

7. FISH AND FISH PRODUCTS
   1. Carp*
   2. Catfish*
   3. Cod***
   4. Fish paste (Kamaboko)***
   5. Flounder**
   6. Herring**
   7. Mackerel***
   8. Norway haddock***
   9. Plaice**
   10. Sea trout*

8. SHELL FISH
   1. Clams**
   2. Shrimps**

9. MEATS[1]
   1. Bacon***
   2. Beef and beef products***
   3. Composite diets containing meat**
   4. Ham***
   5. Horse**
   6. Mixed offal**
   7. Pork***
   8. Poultry***

*** Food item extensively tested with one or more long-term studies available.
** Food item less extensively tested with no long-term study normally available.
* Food item poorly tested.

[1] Some of these studies were carried out at very high radiation doses for specific purposes.

ANNEX 1

A SUMMARY OF TOXICOLOGICAL DATA ON IRRADIATED FOODS

FOOD ITEM	OVERALL AVERAGE RADIATION DOSE (kGy) STORAGE TIME	ANIMAL FEEDING STUDIES		GENOTOXICITY TESTS
		Test/Duration	Animal species	
DATES	0.55-0.8	Short-term toxicity reproduction (1 generation) (98 days)	Rat	
DATES (digests and aqueous extracts)	0.5 0.5			Ames test SCE test Cell survival Cell mutation
DATES (whole dates and digests)	0.55-0.7			Drosophila (sex-linked recessive lethal test)

A SUMMARY OF TOXICOLOGICAL DATA ON IRRADIATED FOODS

FOOD ITEM	OVERALL AVERAGE RADIATION DOSE (kGy) STORAGE TIME	ANIMAL FEEDING STUDIES		GENOTOXICITY TESTS
		Test/Duration	Animal species	
DATES	1		Rat Mice Chinese hamster	Micronucleus test
	1		Mice Chinese hamster	SCE test
	1		Mice	SCE spermatogonia
DATES	0, 6.25, 12.5, 25, 50	Egg development	Oryzaephilus surinamensis (saw-toothed grain beetle)	
DATES	7		Chinese hamster	DNA metabolism bone marrow metaphase analysis
DATES	0, 6.25, 12.5, 25, 50	Egg development	Ephestia cautella (fig moth)	
DATES	1, 2	5 generation rearing	Ephestia cautella (fig moth)	
DATES	1	Development fecundity rearing	Ephestia cautella (fig moth)	

A SUMMARY OF TOXICOLOGICAL DATA ON IRRADIATED FOODS

FOOD ITEM	OVERALL AVERAGE RADIATION DOSE (kGy) STORAGE TIME	ANIMAL FEEDING STUDIES		GENOTOXICITY TESTS
		Test/Duration	Animal species	
MANGO	0.75	1 generation reproduction (10 weeks)	Rat	
MANGO	0.75	2 generation reproduction, teratogenicity, (113 weeks) Short-term (90 days)	Rat Rat	Dominant lethal, chromosome aberration
MANGO	0.8		Chinese hamster	SCE test Micronucleus test
MANGO	0.8			SCE Mutation rate
APRICOTS	2.5		Rat	Dominant lethal
DRIED ONION	0.15 (min) -0.3 (max)	Long-term (3 generations) reproduction teratology (14 weeks)	Rat	Dominant lethal
DRIED ONION	0.1	Short-term reproduction (1 generation) (90 days)	Mice Rat	Dominant lethal
DRIED ONION	0.1		Mice	Micronucleus (bone marrow cells)
ONION (cell sap) (raw and cooked)	0.15, 0.3			Reverse mutation assay (E. coli) DNA repair

A SUMMARY OF TOXICOLOGICAL DATA ON IRRADIATED FOODS

FOOD ITEM	OVERALL AVERAGE RADIATION DOSE (kGy) STORAGE TIME	ANIMAL FEEDING STUDIES		GENOTOXICITY TESTS
		Test/Duration	Animal species	
ONION extract	0.15 (DL) 0.50 (Ames, SCE)		Mice Rat Mice	Chromosomal aberrations (bone marrow cells, mouse) host-mediated assay (G46) 1) Ames test 2) Chromosome breakage 3) SCE (human diploid and Chinese hamster cells) Dominant lethal Micronuclei 4) Gene mutation CHV-79
ONIONS (curry preparation)	0.1			Cytotoxicity and mutagenicity tests (Pseudomonas fluorescence, onion root tips)
ONION POWDER (enzymatic digests and aqueous extracts)	5, 10			Ames test
ONION POWDER (enzymatic digests and aqueous extracts)	0.15, 9.5, 13.6			Ames test
ONION POWDER	0.15, 9.5, 13.6		Chinese hamster Mice	SCE
ONION POWDER	0.15, 9, 15		Chinese hamster	Chromosome analysis (bone marrow cells) DNA metabolism
ONION	0.3			Ames test Chromosome analysis

A SUMMARY OF TOXICOLOGICAL DATA ON IRRADIATED FOODS

FOOD ITEM	OVERALL AVERAGE RADIATION DOSE (kGy) STORAGE TIME	ANIMAL FEEDING STUDIES		GENOTOXICITY TESTS
		Test/Duration	Animal species	
ONIONS (fresh)	0.1	Short-term (3 months)	Rat	Dominant lethal
ONION POWDER (enzymatic digests and aqueous extracts)	15			SCE Forward mutation chromosome
ONION POWDER	0.15, 9.5, 13.6			Drosophila
LEGUME	1.0	Short-term feeding 1 generation reproduction (90 days)	Rat	
LEGUME (White bean)	1.0		Chinese hamster	SCE test Micronucleus test
LEGUME (Black bean)	0, 1, 2	Offspring survival	German cockroach	Chromosomal analysis Dominant lethal
LEGUME (White bean)	10		Chinese hamster	DNA metabolism
LEGUME (Kidney bean)	50	Nutritional studies	Rat	
LEGUME (White bean)	10			SCE Forward mutation Chromosomal analysis (mammalian cells)

A SUMMARY OF TOXICOLOGICAL DATA ON IRRADIATED FOODS

FOOD ITEM	OVERALL AVERAGE RADIATION DOSE (kGy) STORAGE TIME	ANIMAL FEEDING STUDIES		GENOTOXICITY TESTS
		Test/Duration	Animal species	
LEGUME (White and red spotted kidney beans)	0, 0.2, 0.4, 1, 5, 10	Egg development and emergence	Bean weevil (*Z. subfaciatus* *C. annales*)	
LEGUME (Black bean)	0.2, 0.5	Short-term feeding (12 weeks)	Rat	
	0.2, 0.5		Mice	Dominant lethal
OTHER VEGETABLES				
VEGETABLE MIXTURE (leek, celery, carrot, cauliflower) aqueous extract	3.8			Ames test
DRIED VEGETABLE INGREDIENTS				
GARLIC POWDER (aqueous extract)	10 (max)			Ames test
RICE	0.5, 1	Long-term (24 months)	Rat	
	0.5, 1	Long-term (20 months) reproduction	Mice	
		Teratology		
	1	Subacute (24 months)	Monkey	
RICE	1? (not stated)		Mice	Dominant lethal Cytogenetic study (Chinese hamster bone marrow cells)
RICE	0.2, 1, 5, 15	Mortality, fecundity	Weevil	

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FOOD ITEM	OVERALL AVERAGE RADIATION DOSE (kGy) STORAGE TIME	ANIMAL FEEDING STUDIES		GENOTOXICITY TESTS
		Test/Duration	Animal species	
RICE			Mice	Dominant lethal Chromosome aberration (bone marrow) Ames test Mutation assay (Chinese hamster cell V.79) Chromosome aberration (human diploid and Chinese hamster cells)
WHEAT (stored and freshly irradiated)	0.75		Mice	Chromosomal analysis Polyploidy Dominant lethal
WHEAT (freshly irradiated)	0.75		Rat	Dominant lethal
WHEAT (freshly irradiated)	0.75	Immune response (12 weeks)	Rat	
WHEAT (freshly irradiated and stored)	0.75		Monkey	Chromosomal analysis Polyploidy (peripheral lymphocytes)
WHEAT (stored 30 days)	0.2, 2		Mice	Dominant lethal Specific locus test Chromosome analysis (sex cells)
WHEAT (fresh and stored)	0.2, 2	Reproductive performance	Mice	Dominant lethal Chromosomal aberrations (testes) Gonadal cell survival

A SUMMARY OF TOXICOLOGICAL DATA ON IRRADIATED FOODS

FOOD ITEM	OVERALL AVERAGE RADIATION DOSE (kGy) STORAGE TIME	ANIMAL FEEDING STUDIES		GENOTOXICITY TESTS
		Test/Duration	Animal species	
WHEAT			Mice	Dominant lethal Micronucleus Ames test Chromosomal aberration test (human diploid cells HE 2144, Chinese hamster cells Don-6)
WHEAT (freshly irradiated)	0.75		Mice	Dominant lethal Polyploidy (bone marrow cells) Micronucleus test
MAIZE	0.2, 1, 5, 15	Mortality, reproduction	Maize weevil	
POTATO (raw and cooked)	0.1		Mice	Cytogenetic studies (bone marrow cells)
POTATO Chlorogenic acid			Rat	Micronucleus
POTATO	0.1			Cytotoxicity and mutagenicity tests (Pseudomonas fluorescence)
POTATO	0.6			Ames test Chromosome analysis (CHL)
SPICE MIXTURE (at 2, 3, 5, 7.5, 10, 15 and 25% dietary level)	0, 15	Short-term Long-term (90 days, 16 weeks, 2 years)	Rat	
(25%)	0, 15	Short-term toxicity (24 weeks)	Rat	

A SUMMARY OF TOXICOLOGICAL DATA ON IRRADIATED FOODS

FOOD ITEM	OVERALL AVERAGE RADIATION DOSE (kGy) STORAGE TIME	ANIMAL FEEDING STUDIES		GENOTOXICITY TESTS
		Test/Duration	Animal species	
PAPRIKA	50		Mice	Host-mediated assay Ames test
PEPPER, PAPRIKA, SPICE MIX	15	Teratology, reproduction	Rat	
PEPPER, PAPRIKA, SPICE MIX (spice extract and urine metabolites)	5, 15, 45		Rat	Ames test
PEPPER, SPICE MIX	5, 15	Prophage induction	Rat	
PAPRIKA	30		Mice	Micronucleus test
PAPRIKA	15	Preliminary studies, semi-chronic toxicity (215 days)	Rat	Chromosomal analysis (testes, bone marrow)
PAPRIKA	15	Liver function	Rat	
SPICE MIXTURES		Supplement studies (90 days)	Rat	
SPICES	?	Semi-chronic	Rat	
COCOA BEANS	3-5 (max)	Short-term toxicity, 1 generation reproduction (90 days)	Rat	
COCOA BEANS	5	Short-term feeding study (18 weeks)	Rat	

A SUMMARY OF TOXICOLOGICAL DATA ON IRRADIATED FOODS

FOOD ITEM	OVERALL AVERAGE RADIATION DOSE (kGy) STORAGE TIME	ANIMAL FEEDING STUDIES		GENOTOXICITY TESTS
		Test/Duration	Animal species	
COCOA BEANS	3		Chinese hamster	SCE test Micronucleus test
COCOA BEANS				SCE test Forward mutation, chromosome analysis
COCOA BEANS	10		Chinese hamster	DNA metabolism
COCOA BEANS	0.2, 0.4, 1, 5, 10	Egg development (2 months)	Cocoa moth (Cadra cautella)	
COFFEE (from irradiated coffee bean)	0, 0.75, 1	Offspring survival	German cockroach	Chromosomal analysis
COFFEE (from irradiated coffee bean)	0.75, 1	Short-term feeding (12 weeks)	Mice	
COD/REDFISH	1.75	Short-term chronic toxicity (90 days)	Mice	
COD/REDFISH	1.75	1) Multigeneration reproduction 2) Teratology 3) Carcinogen (80 weeks)	Mice	Dominant lethal
COD/REDFISH	1.75	Short-term (9 months)	Dog	

A SUMMARY OF TOXICOLOGICAL DATA ON IRRADIATED FOODS

FOOD ITEM	OVERALL AVERAGE RADIATION DOSE (kGy) STORAGE TIME	ANIMAL FEEDING STUDIES		GENOTOXICITY TESTS
		Test/Duration	Animal species	
COD/REDFISH	1.75	Short-term: serum alkaline phosphatase evaluation (16 weeks)	Rat	
FLOUNDER (yellow-tailed)	1.75	Short-term 1 generation reproduction (90 days)	Rat	
PLAICE (European)	1.75	Short-term 1 generation reproduction (90 days)	Rat	
MACKEREL (Indian)	1.5	Short-term 1 generation reproduction (90 days)	Rat	1) Dominant lethal 2) Bone marrow metaphase analysis 3) Host-mediated assay
MACKEREL (Indian)	1.5		Mice	Dominant lethal assay Micronucleus test
REDFISH	2	Liver enzyme analysis (42 days)	Rat	
COD (digest and extract)	2.2 2.2			Ames test 1) Sister chromatid exchange 2) Cell survival, cell mutation
COD	2.5		Rats, mice, Chinese hamster	Micronucleus test Sister chromatid exchange test Spermatogonia test

A SUMMARY OF TOXICOLOGICAL DATA ON IRRADIATED FOODS

FOOD ITEM	OVERALL AVERAGE RADIATION DOSE (kGy) STORAGE TIME	ANIMAL FEEDING STUDIES		GENOTOXICITY TESTS
		Test/Duration	Animal species	
COD	2.5		Chinese hamster	DNA metabolism
COD	2.5		Chinese hamster	Bone marrow metaphase analysis
CARP	2.5 2.5 2.5	Short-term (3 months)	Mice Mice Mice	Dominant lethal Host-mediated assay
COD	3, 6, 12			Ames test
COD/CARP/ CATFISH (fresh)	2	Long-term 4 generation reproduction, teratology (29 months)	Rat	1) Dominant lethal assay 2) Chromosome analysis (bone marrow)
COD/SPRAT (hot-smoked)	2	Short-term reproduction (1 generation) (7 months)	Rat	
MACKEREL (salted-dried)	2	Long-term reproduction (2 generation) Teratogenicity (104 weeks)	Rat	Dominant lethal
TROUT		Short-term	Rat	
FISH	2			Cycotoxicity (onion root tip cells)
HERRING FILLETS (saline and ethanol extracts)	3, 6, 12			Ames test

A SUMMARY OF TOXICOLOGICAL DATA ON IRRADIATED FOODS

FOOD ITEM	OVERALL AVERAGE RADIATION DOSE (kGy) STORAGE TIME	ANIMAL FEEDING STUDIES		GENOTOXICITY TESTS
		Test/Duration	Animal species	
MEATS				
BEEF	47-71			Drosophila (sex-linked recessive lethal test; chromosomal analysis)
HAM	37-42			Drosophila (sex-linked recessive lethal test; chromosomal analysis)
BEEF (irradiated raw, then cooked)	6		Rat	Dominant lethal
HAM (raw pork product)	2	Long-term feeding 2 generation reproduction (2 years)	Rat	
CHICKEN				
CHICKEN digests and aqueous extracts	7			Ames test SCE test Cell survival Cell mutation
CHICKEN	7		Rat, Mice, Chinese hamster	Micronucleus test
			Mice, Chinese hamster	SCE test
			Mice	Spermatogonia test

A SUMMARY OF TOXICOLOGICAL DATA ON IRRADIATED FOODS

FOOD ITEM	OVERALL AVERAGE RADIATION DOSE (kGy) STORAGE TIME	ANIMAL FEEDING STUDIES		GENOTOXICITY TESTS
		Test/Duration	Animal species	
CHICKEN (frozen, thermal, gamma- and electron-irradiated)	10 Mev, 45 kGy (radappertising dose)			Ames test
CHICKEN (frozen, thermal, gamma- and electron-irradiated)	10 Mev, 45 kGy (radappertising dose)	Also, dose response, fecundity, egg viability		Drosophila (sex-linked recessive lethal test)
CHICKEN	7		Chinese hamster	DNA metabolism Bone marrow metaphase analysis
CHICKEN (frozen, thermal, gamma- and electron-irradiated)	10 Mev, 45 kGy (radappertising dose)	Long-term reproduction (32-36 months) Long-term teratology 3 generation reproduction (-24 months) PER study	Dog Mice Hamster and Mice Rat	Dominant lethal (mice)

ANNEX

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