THE ROLE OF THE INTERNATIONAL FOOD IRRADIATION PROJECT IN WHOLESOMENESS STUDIES

P.S. ELIAS

International Food Irradiation Project, Federal Research Centre for Nutrition, Karlsruhe, West-Germany

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 U.S. Naturally concern also arose over the wholesomeness of food preserved in this manner and in the light of the legal approach required by the U.S. Food Legislation it was 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 rationalise and coordinate these various efforts in a more economic fashion the International Project in the Field of Food Irradiation was set up 10 years ago as a result of an agreement between 19 interested countries under the joint sponsorship of the IAEA (International Atomic Energy Agency of UNO), the FAO (Food and Agricultural Organization of UNO) and the NEA (Nuclear Energy Agency) of the OECD (Organisation for Economic Cooperation and Development) (1). Its objectives were essentially the carrying out of a modest research programme into methodology and the coordination, including supervision, of wholesomeness testing and related studies in laboratory animals, contracted out to reputable laboratories on behalf of the member countries. Additionally, the dissemination of information concerning wholesomeness testing of irradiated foods and assistance to authorities in their consideration of acceptance of irradiated food constituted further objectives (1).

As a result of the effective operation of the Project some 12 feeding studies were placed with contracting laboratories to investigate various toxicological aspects of irradiated wheat, flour, potatoes, rice and fish, in line with the recommendations of the 1969 Joint Expert Committee (2). IFIP also attempted to alter the direction of the animal investigations towards the clearance of groups of similar foods and to provide basic information on the toxicological effects of variations in the composition of irradiated laboratory animal diets. IFIP soon recognized that the vast amount of data available on the radiation-induced chemical changes in food components could be used in the evaluation of the wholesomeness of irradiated foods. The publication of a monograph collecting together critical reviews of the radiation chemistry and the yields of radiolysis products of major food components was another important step towards achieving an easing of the burden of innumerable toxicological investigations of individual irradiated foods (3).

The outcome of the 1976 Joint FAO/IAEA/WHO Expert Committee (JECFI (4) confirmed indeed the value of the reorientation in the approach to assessing the wholesomeness of irradiated foods initiated by the International Project. Apart from acknowledging the need to consider food irradiation as a process, analogous to other more traditional physical treatments for preserving food, JECFI recognized that the evaluation of the wholesomeness of foods processed by this technology posed problems different from those encountered with food additives or contaminants. The 1976 JECFI went even as far as envisaging, that in the future radiation chemical data alone, in conjunction with all other available evidence from animal feeding studies, might suffice to conclude that foods processed by irradiation with doses up to 5 kGy were safe for consumption by man. Furthermore, the 1976 JECFI gave unconditional acceptance to 5 irradiated foods and provisional acceptance to another 3 irradiated foods. This opened the way for developing Draft General Standards on Irradiated Foods and a Draft Code of Practice for the Operation of Irradiation Facilities Used for the Treatment of Foods under the Joint FAO/WHO Food Standards Programme through the machinery of the Codex Alimentarius Commission (5). The objectives of Codex Alimentarius Standards are the removal of barriers to international trade in foods for which such Codex Standards have been elaborated, the standards having been incorporated into their national laws by the 120 member states of the Codex Alimentarius Commission. Clearly industrial interest in developing this technology could not be expected to arise, unless there existed a definite possibility for unhindered international trade in irradiated food commodities.

After this encouraging turn of events the Project decided to reorientate its entire programme (6). The main thrust now aimed at achieving acceptance of the process of irradiation for food preservation up to average doses of 10 kGy creating no more toxicological problems than any of the other traditional preservation processes eg. pasteurization, canning, deep-freezing, microwave-heating etc. It had been accepted previously (4) that irradiation-preserved foods presented no specific microbiological or nutritional hazards compared to any other physically or chemically preserved foods, provided proper hygienic requirements similar to those appropriate to traditionally preserved foods were observed in handling such irradiation-preserved foods.

To obtain this general clearance of the irradiation process it was felt necessary to assemble for consideration by the International Expert Committee a body of scientific evidence which would validate such a decision. The cornerstone of the argumentation would rest on convincing evidence from the radiation chemistry of food components, supporting the validity of any extrapolation of safety data from animal feeding studies within major classes of foods and between various classes of foods. To gather this evidence a coordinated programme on the radiation chemistry of food and food components (CORC programme) was set up in 1978 (7). This programme attempted to channel the research activities of nine laboratories specialising in radiation chemical investigations toward providing the unified body of evidence identified by the 1976 Joint Expert Committee. An important part of the programme was devoted to the review of recent data on the identification and quantitative measurement of radiolytic products as well as the provision of comparative data on the effects of radiation and conventional methods of food processing.

To date it has been possible to study, on a comparative basis, the radiolysis products from various starches, the uniformity of protein and lipid radiolysis in various meats, and the reaction mechanisms in irradiated fruits. Although these studies dealt with considerably different systems and approaches, they offered strong evidence of uniformity, predictability and the ability to extrapolate. They demonstrated that even in complex foods the nature of the radiolysis products from the individual food components was the same and that the yields of these products were determined mainly by the concentration of the precursor components and the radiation dose. Thus foods with like chemical composition yielded a similar spectrum of radiolysis products of predictable nature and maximum possible yields. Therefore foods could be grouped confidently into a few broad classes, within which the results of animal feeding studies or mutagenicity tests could be extrapolated from individual foods to the remainder of the class. Similarly, results obtained under one set of irradiation conditions could be extrapolated to others without the need for further biological testing. This strengthened the suggestion, already anticipated by the 1976 Joint Expert Committee, that radiation chemical evidence could play an even greater role in the evaluation of the safety of irradiated foods than was previously thought likely (8).

An interesting development of the attention paid to radiation chemistry of foods was the development of a theoretical model which would permit the application of competition kinetics to the radiolytic events occurring in a living cell and subsequent confirmation of the predicted yields of radiolysis products by analysis of actual irradiated food (9). Such a model was developed for irradiated fruits and its usefulness demonstrated by the close agreement obtained between the nature and yield of the radiolysis products, calculated by means of a suitable computer programme, with the actual analytical results obtained on irradiated fruit juice, fruit pulp and whole fruits (9).

During the early years of the Project first priority was given to the carrying out of wholesomeness testing by extensive and expensive animal feeding studies on a contract basis for the benefit of member countries. These studies were devoted to fulfilling the requests of earlier Joint Expert Committees which had given provisional clearances only to a very few irradiated foods i.e. potatoes, wheat and flour. Additional foodstuffs chosen by IFIP for study by animal feeding test were fish, rice, spices, mango, dried dates and onions. The selection of the foodstuff was based on 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 about the 10 kGy range (6).

The methods used for wholesomeness testing varied somewhat from study to study, but all involved the incorporation of the irradiated or non-irradiated foodstuff into the diet of laboratory animals. Groups of animals fed on such diets are observed over most of their life span, and their health, growth, development and reproductive capacity compared with that of control animals receiving a diet containing the unirradiated foodstuff. Some of these studies were relatively short, extending over 90 days and one reproductive cycle, others continued for 2 years or longer and involved up to four filial generations. Many different factors have had to be taken into account in assessing the effects of the irradiated food, including rate of growth, incidence of disease including cancer, changes in haematological and biochemical parameters, reproductive capacity, fertility, and abnormalities in the offspring. At the end of the tests tissues were examined microscopically for signs of disease (6).

In none of these studies, nor in any properly conducted studies published in the literature, were any significant effects observed which could be attributed solely to the irradiation treatment received by the food administered to the laboratory rodents used in these tests. Several drawbacks inherent in this approach to evaluation of the wholesomeness of irradiated foods require discussion. The toxicological testing of irradiated food presents rather special problems in comparison to the testing of food additives or contaminants (10). Firstly, there is the difficulty of specifying precisely the material to be examined. This is comparatively easy in the case of a single defined chemical entity, but becomes extremely complicated where a complex food is concerned, subject to wide compositional variations both seasonal and geographical. It is also impossible to incorporate sufficient of the irradiated food into the diet of the laboratory animals in order to achieve a satisfactory margin of safety without considerable disturbance of their nutritional requirements. Moreover, any imbalances of the major components of the diet of laboratory animals influences profoundly the natural background of disease and tumor incidence in the animals under test. Finally, unless the toxicologically significant radiolytic product in the irradiated food possessed extremely potent toxic properties, its concentration in the irradiated food usually would be so low as to be practically undetectable by a conventional design employing groups of 50-100 animals per test group. This naturally raises the question of the appropriateness at least of long-term animal feeding tests based on conventional protocols.

Radiation chemistry data had already revealed that breakdown products from radiolysis were formed at levels in the part per million range or lower and very rarely exceeded 100ppm (11). At these levels much larger numbers of animals would have to be used in each experimental group in order to permit appropriate statistical evaluation of any observed differences from controls. The impossibility was also realized of being able to test every individual food which might be suitable for preservation by irradiation. This handicap was due to the costs involved, the scarcity of adequate laboratory facilities throughout the world equipped to perform these investigations under acceptable good laboratory practice, and the already existing demand for their services consequent upon safety legislation related to industrial and environmental chemicals.

Recognition of the likely validity of these arguments and the precipitous escalation of the costs of animal feeding studies, together with the assertion by the 1976 Expert Committee that the use of animal feeding tests as the mainstay of wholesomeness evaluation of irradiated foods was inappropriate (4), enabled the Project to abandon this unprofitable exercise. Realising that only a few selected animal studies might be needed in future for wholesomeness evaluation purposes, the Project commissioned combined 90-day-one-generation-reproduction studies on cocoa beans and legumes as the only other animal feeding tests under its scientific programme. However, information derived from the use of irradiated diets for the large-scale production of laboratory rodents (12) and from the use of irradiated feeds in the husbandry of farm animals (13) appeared to offer useful ancillary evidence for the wholesomeness of irradiated foods. Hence critial reviews of these subject areas were commissioned by the Project.

The nutritional aspects of the wholesomeness of irradiated foods were not expressly included in the scope of the activities of the International Project (1). The major problem of the use of ionizing radiation on food is related to the destruction of certain nutrients to a variable degree, particularly certain vitamins e.g. B_1, E and, to a lesser extent, C. Decreases in the vitamin or other essential nutrient content of irradiated foods are not significant, where these foods form only a small proportion of the total diet. In circumstances where these foods constitute a major portion of the diet, and thus are vectors of these essential nutrients, appropriate supplementation of the diet would be necessary (4).

Similarly, the microbiological aspects of the wholesomeness of irradiated foods were not directly part of the activities of the International Project (1). However irradiation of food is an important measure for removing pathogenic organisms, particularly food poisoning bacteria of the genus Salmonella. If applied at an appropriate dose rate, it is possible either to sterilize food or to reduce the total viable count. No evidence has ever been produced that irradiation at the levels used induced mutation of non-pathogenic organisms into pathogenic forms (14). Similarly no evidence has been advanced that irradiation is able to change the character of food-borne viruses, although it is probable that this form of processing does not eliminate viruses from food.

Specifically included in the scope of activities of the Project was a modest programme of in-house research into methodology (1). The need for looking at the options for improving the toxicological testing procedures, existing at the time of the formation of the International Project, was demonstrated by the enormous variability in the testing requirements of international expert committees and national authorities, when called upon to evaluate the wholesomeness of irradiated foods. To eliminate these problems considerable attention was devoted initially by the Project to designing elaborate protocols for animal feeding studies. In the Project's own laboratory the effects of irradiated food on the immune system of the rat were studied as a possible sensitive indicator for biologically active radiolytic products in irradiated foods (15). Other in-house investigations before 1976, though largely unsuccessful, aimed at developing a suitable semi-synthetic diet for use in long-term animal feeding tests investigating irradiated fatty foods.

The advent of a number of simple, short-term, sensitive tests for mutagenic potential, which later had been shown also to correlate closely with carcinogenic potential, opened up new possibilities for the screening of irradiated foods for the possible presence of mutagenic or carcinogenic radiolytic products. A large spectrum of in-vitro and in-vivo procedures now exists for screening for mutagenic potential (16). The Project investigated these new test systems and established an acceptable methodology capable of validation and showing good reproducibility (17). Furthermore, a comprehensive programme of mutagenicity testing was instituted by the Project, involving many different methods and a large variety of irradiated foods. Incidentally, the methodology developed by the Project for testing irradiated foods by short-term mutagenicity screens has applications beyond the wholesomeness aspects of irradiated foods. The methodology is of a general nature and can be used to investigate the effects of any form of food processing on the toxicological qualities of the processed food (18).

Briefly, the new method of sample preparation by enzymatic digestion in-vitro was developed in an attempt to overcome some of the drawbacks associated with the use of solvent extracts or natural juices prepared from the irradiated food by physical means. The latter samples were used hitherto to circumvent the problem of testing processed foods most of which are unsuitable for direct incorporation into in-vitro test systems. The use of extracts is open to criticism despite its undoubted value because a) it is very difficult to extract all possible process-induced toxic compounds from all foods, b) macromolecular scavenging might interfere with the extractability of relevant compounds, c) active mutagens might be destroyed during normal digestion, so that activity noted in extracts might have little relevance to the effects of food on an intact organism, d) reactions might occur between food constituents and solvents. The digestion method involves digestion of an aqueous food homogenate at acid pH by pepsin and at alkaline pH by pancreatin, centrifugation, and sterilisation by ultrafiltration. The sterile digests are then used directly in the various in-vitro mutagenicity tests (19).

The in-vitro procedures selected by the Project included the Salmonella bacterial mutation test (Ames test) with and without metabolic activation, point mutation assays in cultured mammalian cells, SCE assays in cultured mammalian cells, mammalian cell transformation assays, determination of unscheduled DNA synthesis and investigation of DNA repair. The in-vivo procedures included a micronucleus test, an SCE test in bonemarrow and spermatogonia, and the induction of recessive lethals in Drosophila. The in-vitro studies were carried out on the same extracts and digests in four different institutes including the Project's own laboratory, while the in-vivo studies were based on the same irradiated foods but were performed in two separate institutes.

Short-term mutagenicity screening has been carried out so far on irradiated and non-irradiated chicken, fish, dried dates, beans, cocoa beans, mangoes, dried onions and a mixed diet. Spices were also investigated as an "in kind" contribution to the Project by one member country. The screening procedures chosen for spices were the Salmonella bacterial mutation test (Ames test), the lambda-prophage induction test, the induction of recessive lethals in Drosophila and a host-mediated assay. None of these sensitive screening tests have produced any evidence for the presence of mutagenic or carcinogenic radiolysis products in the irradiated foods investigated. These findings agree closely with the results of the animal feeding tests carried out on similar foods or related food materials processed by irradiation.

Many studies on irradiated foods exist which have not yet been considered by a Joint FAO/IAEA/WHO Expert Committee on the Wholesomeness of Irradiated Foods. Of particular interest to this Congress are of course studies related to meat and meat products. The 1976 Joint Expert Committee gave a full clearance to eviscerated prepacked chicken, irradiated at 5-7 kGy for the elimination of pathogenic microorganisms (4). The real hazard from prepacked frozen chicken is due to contamination with Salmonella. There are no satisfactory alternative procedures available at the moment to eliminate this contaminant. Great care has to be exercised when handling broiler chicken. It has to be cooked adequately, otherwise disease due to Salmonella infection is likely to occur. In part this microbial contamination arises from the use of contaminated feeding stuffs, which have not been treated specifically to eliminate microbial contaminants.

At present the USSR has approved experimental batches of eviscerated poultry, packaged in polythene bags, irradiated at 6 kGy (4.7.66), but their marketing experience is unknown.

The Netherlands fully approved for sale dry slaughtered broiler chicken (20) aged 10 weeks, parts of chicken and separately packaged heart, liver, stomach and neck, if irradiated with a maximum dose of 3 kGy ± 5%; the conditions attaching to this clearance (10.5.76) were:

a) the period between slaughter and irradiation not to exceed 24 hours;

b) the plastic packaging to be of the FDA approved type;

c) the pack to be marked specially for sale by a specific sign and to be labelled with keeping date;

d) to store the packaged food at 2-5°C;

e) to review the situation in 5 years.

This approval was based on the decision of the 1976 Joint Expert Committee and on microbiological evidence that less than 10 organisms survived irradiation treatment, that keeping and storage were improved, that the biological value of the protein remained unchanged, that little evidence of amino acid destruction was seen and that the vitamin content was not changed significantly.

Canada also approved the sale of irradiated chicken for test marketing; irradiation was to be at maximum 7 kGy (20.6.73). However, no market testing has been carried out so far.

South-Africa has recently approved the sale of irradiated chicken treated over a dose range of 2-7 kGy(August 1978) based on the acceptance by the 1976 Joint Expert Committee of the wholesomeness of chicken so processed (20).

In the U.S.A., on the basis of a special FDA exemption, diet portions of radiation-sterilized chicken breast, pork link sausage, pork chops, bacon, ham, beef steaks, and ground beef patties have been provided to the Fred Hutchinson Cancer Centre in Seattle, WA for patients requiring sterile foods (Radiation Technology, Inc.) since 1968. These portions were packaged in retortable pouches. Here, the condition for treatment is sterilization at 24-43 kGy. This is a comparatively large dose, but the products are irradiated at -30°C. Over the past 5 years of use these patients who are probably the most vulnerable subjects because of their lack of immune defences, have been kept alive and well on irradiated sterilized food (21).

A petition for clearance of radurized poultry has been submitted to FDA by Radiation Technology, Inc. It is currently under active review. The FDA is thus once again forced into the position of having to consider a submission on this subject. It will be interesting to see the outcome. However, it is likely that no decision will be forthcoming for the next 2-3 years, because the American research group in "Natick" is conducting extensive studies on irradiated chicken and chicken products involving several feeding studies on chicken irradiated with higher doses, for sterilization for army use. Clearly no decisions will be taken until the results of all these tests are to hand. It is quite possible that in some other countries submissions will come forward, probably also in the U.K., for the clearance of chicken treated by radiation specifically against Salmonella contamination.

In the Netherlands limited market trials have been carried out in 1976 with irradiated fresh broilers. With the assistance of a major national wholesale company this commodity was distributed to four hospitals and an old peoples' home for evaluation by purchasers, dietitians and kitchen personnel. The meat was broiled or cooked on the 4th, 8th and 10th day after slaughter. The prepared broilers scored highest on the 8th day and the lowest on the 4th day, where 82.3% of the participants marked this food as "excellent" to "good" and 17.7% as "fairly good". No marks for unacceptability were recorded. None of the participants had any objections to the fact that the food had been irradiated and indeed the improved hygienic (microbial) quality was greatly appreciated (22).

Market trials of this nature will shortly be carried out on an expanded scale since full commercialisation of this product is planned; this appears possible because an unconditional Ministerial "clearance" is now available.

In 1976 Kahan and coworkers reported from the U.S. Natick Laboratories that fresh eviscerated broilers could be treated with 2.5 kGy and stored for 15 days at 1.6°C without deterioration. The birds were found to be free from Salmonella (23).

At present the U.S. Army Natick Research and Development Command are conducting an extensive animal testing programme on chicken either gamma-irradiated in metal cans or electron-irradiated in flexible pouches. The irradiation dosage is 45-69 kGy at -30°C. The meat is being fed to rats, mice and dogs as deboned ground meat and skin. Three years' studies were carried out in rats and mice and a two year study in dogs. A multigeneration study was done in dogs but difficulties arose with reproduction on this diet. In addition mutagenicity studies in Drosophila and a heritable translocation test in mice are under way. The latter is probably one of the most expensive studies because something like 30,000 progeny of mice have to be followed over six generations. An Ames (Salmonella reverse mutation) test was also performed. The results were negative (24). This fits in well with the studies contracted through the Project. These latter studies included the investigation of water extracts and digests of irradiated cooked chicken by the Ames test, by forward mutation tests and chromosomal aberration tests in-vitro in cultured cells, by in-vivo tests in rats, mice and hamsters fed on irradiated chicken and by studies on the induction of DNA damage. The latter studies were carried out at an institute in Vienna. Again there was no evidence that feeding irradiated chicken induces any mutations in the various test systems or causes damage to the DNA.

Despite earlier setbacks, the U.S. Army Natick Research and Development Command continued successfully in devising a process resulting in good quality radiation-sterilized beef highly stable on storage. Renewed wholesomeness studies using animal feeding studies were started in 1971 using irradiated and non-irradiated beef and comparing additionally gamma-irradiation with electron irradiation and heat-sterilization with frozen storage. These studies were completed early in 1977 and the petition is with the FDA since the fall of 1977. Further long-term animal feeding studies on pork and ham were started at the end of 1976 and are expected to be completed by 1981, in which year further petitions for the clearance of irradiated pork and ham are to be submitted to the FDA (25).

A further, and particularly promising application of irradiation is in the preservation of bacon, where long shelf-life can be obtained without using nitrites. Other advantages of irradiation in bacon production are the possibility of reducing the addition of nitrite for colour purposes by over 80% and of salt by 50% in prefried bacon. Moreover, irradiated bacon contained no residual nitrite compared to commercially cured samples and on frying significantly less nitrosopyrrolidine was formed in irradiated bacon compared with non-irradiated control samples.

It is to be hoped that the 1980 Joint FAO/IAEA/WHO Expert Committee will accept irradiation as a preservation process for food causing no toxicological problems, if applied under strictly controlled conditions over standardised dose ranges. If this is then followed by approval by the U.S. authorities of the safety for marketing irradiated chicken, beef, pork, and ham, the doors will be open to the industrial application of irradiation technology on a commercial basis for the preservation of any food to which this form of processing is applicable.

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