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WHOLESOMENESS OF IRRADIATED FOOD WITH SPECIAL REFERENCE TO WHEAT, POTATOES AND ONIONS

Report of a Joint FAO/IAEA/WHO Expert Committee

Geneva, 8-12 April 1969

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JOINT FAO/IAEA/WHO EXPERT COMMITTEE ON WHOLESOMENESS OF IRRADIATED FOOD

A Joint FAO/IAEA/WHO Expert Committee on Wholesomeness of Irradiated Food met in Geneva from 8-12 April 1969. The meeting was opened by Dr P. Dorolle, Deputy Director-General of the World Health Organization, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations, the International Atomic Energy Agency, and the World Health Organization.

1. INTRODUCTION

1.1 Terms of reference

Much of the work of the Committee was carried out in separate meetings of the members invited by WHO and those invited by FAO and IAEA. The former had the task of assessing the wholesomeness of certain irradiated foods (i.e., wheat irradiated for disinfestation, and potatoes and onions irradiated to control sprouting), and the latter discussed the technical aspects and provided certain additional information required by the former. (The decisions of the members invited by WHO on the wholesomeness of irradiated foods were based entirely on toxicological and related information, and on a consideration of the possible nutritional implications. Technological and economic aspects were not considered.) In addition, the Committee as a whole discussed testing procedures and future research required for the assessment of the wholesomeness of irradiated food.

1.2 Principles

For the purpose of assessing the wholesomeness of irradiated foods, the Committee agreed to adopt the principles described in the report of the Joint FAO/IAEA/WHO Expert Committee on the Technical Basis for Legislation on Irradiated Food (hereafter referred to as " the report of the 1964 meeting ").[1] The Committee also adopted the general principles

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1966, No. 316; *FAO Atomic Energy Series*, 1966, No. 6.

and approaches laid down in the second, fifth and eleventh reports of the Joint FAO/WHO Expert Committee on Food Additives,[1] and in the report of a WHO Scientific Group on Procedures for Investigating Intentional and Unintentional Food Additives.[2]

Any consideration of the safety-for-consumption of radiation-processed food must be based upon an evaluation of (*a*) the absence of harmful microorganisms and microbial toxins, (*b*) its nutritional adequacy,[3] and (*c*) the absence of any significant toxic products formed in the food as a result of irradiation.

The Committee agreed that at the dose levels required for sprout control of potatoes and onions, or for disinfestation of wheat or wheat flour, microbiological hazards are not a serious consideration. On the other hand, repeated irradiation of certain resistant species of insect could conceivably further increase their resistance; radiation plants should therefore be designed in such a way as to minimize the chance of insects being repeatedly irradiated.

1.3 Extrapolation of data

In evaluating the safety of irradiated wheat and potatoes, the Committee was faced with the problem of having to extrapolate from data obtained under one set of conditions to another set of conditions; it also had to take into account that the form of the irradiated food as eaten by man is different from that in which it had been consumed by animals in the toxicological studies. This problem was considered by the 1964 meeting;[4] an account of the principles adopted by the present Committee in extrapolating data is given in sections 1.3.1–1.3.2 below.

1.3.1 *The validity of data obtained on foods irradiated at high doses for evaluating the safety of foods irradiated at low doses*

The relationship between radiation dose and the chemical and biochemical effects of irradiation on food has not been clearly established. In general, it is known that the concentration of radiolytic products does not necessarily increase linearly as the dose; indeed, there is evidence to suggest that the concentration of radiolytic products may increase with increasing dose until a certain level is attained and that the concentration

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1958, No. 144; 1961, No. 220; 1968, No. 383 (*FAO Nutr. Meet. Rep. Ser.*, 1958, No. 17; 1961, No. 29; 1968, No. 44).

[2] *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 348.

[3] For a discussion of " nutritional value ", see section 2.2 of the report of the 1964 meeting (*Wld Hlth Org. techn. Rep. Ser.*, 1966, No. 316; *FAO Atomic Energy Series*, 1966, No. 6).

[4] See sections 4.3.2 and 4.3.3 of the report of the 1964 meeting (reference given in note 3).

may then remain at this plateau, or even decrease as the radiation dose is increased. Again, owing to the influence of protective mechanisms, a threshold value may exist below which certain changes cannot be detected. Because of such considerations, the Committee was of the opinion that toxicological evidence obtained in animals fed food irradiated at much higher dose levels than those to be used in practice might not be entirely appropriate for the evaluation of the safety of food irradiated at lower doses.

Therefore, in its evaluation of irradiated wheat, the Committee based its decision primarily upon the data from animals fed wheat irradiated at 200 000 rad or less; at the same time, data from high-dose experiments were carefully examined for indications of effects to be particularly looked for in tests conducted with wheat irradiated at low doses.

1.3.2 *The applicability of evidence on safety to all varieties of the same crop, grown under different geographical conditions*

Although quantitative differences in composition are apparent in different varieties of a crop, or in the same crop grown in different areas, the basic composition of the crop is likely to be qualitatively similar. Recognizing the impracticability of requiring evidence of wholesomeness for many varieties, and to take into account regional differences, the Committee therefore decided that for irradiated wheat and potatoes its evaluations should have general applicability to all crops, regardless of the variety or the area in which the crop is grown.

1.3.3 *The applicability of evidence on safety to the same food irradiated in a different form*

The Committee considered the appropriateness of data obtained from animals fed irradiated flour in assessing the safety of irradiated wheat. It was recognized that differences in physical form could influence the radiation-induced chemical changes through factors such as permeability to oxygen. However, the Committee was of the opinion that such differences were likely to be quantitative rather than qualitative, so that data obtained on both wheat grain and wheat flour should taken into consideration and the evaluation would be applicable to both forms, i.e., wheat grain and ground wheat products.

1.4 Mutagenicity and cytotoxicity

Since the report of the 1964 Joint FAO/IAEA/WHO Expert Committee[1] was published more information has become available to show that

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1966, No. 316; *FAO Atomic Energy Series*, 1966, No. 6.

cytotoxic and mutagenic substances may be formed in radiation-processed food. If mutagenic compounds are formed on irradiation, it can be expected that both gene mutations and chromosome aberrations may be induced. *In vitro* and *in vivo* tests are being developed to assess the mutagenic properties of these substances (see Annex 1, p. 16). Of the findings from these two testing procedures, those from *in vivo* tests would be more relevant to the evaluation of possible adverse effects.

The Committee considered the available evidence on possible mutagenicity of irradiated foods, including wheat, potatoes and onions, but in view of the paucity of the data and the uncertainty of their extrapolation to man, the Committee discounted this evidence when reaching its decisions on the wholesomeness of specific irradiated foods. Furthermore, the Committee felt that mutagenic substances might also result from other food processes and that certain food additives and other materials might also have these properties.

1.5 Relationship of the Committee to the Joint FAO/WHO Food Standards Programme

The Committee was informed that the Joint FAO/WHO Food Standards Programme (Codex Alimentarius) would be dealing with the acceptability of specific irradiated foods, taking into consideration the decisions recorded in this report. At meetings of the Codex Committee on Food Additives priorities would be established for those irradiated foods to be considered by future expert committees.

2. INVESTIGATIONS REQUIRED FOR ASSESSMENT OF THE SAFETY OF IRRADIATED FOOD

2.1 Introduction

In order to provide guidance for those conducting investigations into the safety of irradiated food, the Committee critically examined the technical procedures and tests described in section 4 of the report of the 1964 Joint FAO/IAEA/WHO Expert Committee;[1] it was agreed that basically the methods recommended are still valid, but certain modifications and elaborations are proposed below.

The Committee endorses the opinion expressed in the report of the 1964 meeting[1] that the principles of food additive testing are inapplicable to irradiated foods in as far as physical and chemical identification is concerned.

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1966, No. 316; *FAO Atomic Energy Series*, 1966, No. 6.

The Committee re-emphasized the need for collaboration between the appropriate government authorities and investigators who are planning and conducting research to demonstrate the safety of irradiated food, as described in section 4.2 of the report of the 1964 meeting.[1]

2.2 Experimental design

The Committee believes that when long-term investigations are conducted there is considerable merit in the use of a positive control group fed a known carcinogen to check the response of the species used, wherever this is feasible. However, the need for such an additional control group, and the choice of an appropriate carcinogenic agent, should remain at the discretion of the investigator.

In some cases consideration should be given to the use of several control groups; for example, as well as a control group fed the specific food unirradiated, an additional group fed the same food treated by alternative accepted methods, such as thermal processing, might yield useful comparative information.

2.2.1 *Animal species*

The Committee questioned the suitability of chickens for use in long-term feeding tests, since there is insufficient toxicological experience with this species. In carcinogenicity studies, in particular, the use of rats and mice would be preferable to the use of chickens. Recent experience with the miniature pig is not encouraging. There is an urgent need for the discovery of other non-rodent species suitable for long-term studies.

2.2.2 *Numbers of animals*

In the past, feeding studies on the larger species (e.g., dogs and pigs) have often involved too few animals. When larger animals are used, the number of animals in each group should be a minimum of eight; both male and female animals must be used in each group.

2.2.3 *Diets*

Many long-term feeding studies on irradiated foods have produced poor results because insufficient attention has been given to the nutrient content and/or caloric value of the diets used. The diets fed to the various groups should be isocaloric, and of adequate caloric density for the test species involved. Nutrient levels in the diets should be approximately

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1966, No. 316; *FAO Atomic Energy Series*, 1966, No. 6.

equal to the normal requirements for the species and, preferably, should be checked at regular intervals during the course of the feeding experiment.

It is desirable that before the irradiated diet is fed to the test animals it should be subjected to the same conditions and procedures as envisaged in practice and that its effect should be compared with that of a diet treated in an identical manner but without irradiation. Unfortunately, this is not always possible. The Committee stressed that any additional process applied to the control diet must also be applied to the irradiated diet.

When reporting the conditions of the radiation process used to treat the food to be used in feeding tests, it is essential that the radiation source, the energy, the dose of radiation, and the dose rate should be given, together with an adequate description of dose variation in the food. Furthermore, full details of the environmental conditions during and following irradiation should be stated.

2.2.4 *Reproduction*

Experience using dogs has shown that this species is generally unsuited for routine studies of reproductive function. Reproduction studies on two species are required; rats and mice are acceptable and it has been reported that Japanese quails are useful in certain instances.[1]

The teratological examinations recommended in the 1966 report[2] can be conveniently undertaken by breeding third litters in the multi-generation test (other than in the parent generation, which is normally continued for a long-term study) and terminating pregnancy a few hours or a full day before expected delivery so that all surviving fetuses can be examined, and the resorption sites and number of dead fetuses counted, as recommended by the WHO Scientific Group on Principles for the Testing of Drugs for Teratogenicity.[3]

3. EVALUATION OF IRRADIATED FOODS

3.1 Categories of acceptance

In order to make an evaluation of an irradiated food, the Committee examined the extent and quality of the biological evidence and other available data relating to the wholesomeness of the irradiated food. The usual consumption of the food in different countries and its role in nutrition were also considered.

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[1] Padgett, C.S. & Ivey, W.D. (1960) *Anat. Rec.*, **137**, 1.

[2] *Wld Hlth Org. techn. Rep. Ser.*, 1966, No. 316; *FAO Atomic Energy Series*, 1966, No. 6.

[3] *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 364.

The Committee decided that irradiated foods could be placed in three categories of acceptance, broadly similar to the three types of acceptable daily intakes adopted for food additives (described in the eleventh report of the Joint FAO/WHO Expert Committee on Food Additives).[1] The main difference is that in the case of irradiated foods the concept of acceptable daily intake, based upon the daily dose of a chemical that appears to be without appreciable risk, is not applicable, since it would obviously be unnecessary and impracticable to express the results in terms of the precise quantity of a food that could be safely consumed.

The categories of acceptance are :

(*a*) *Unconditional acceptance,* granted where adequate biological data are available for the establishment of safety, including the results of long-term toxicological investigations with relevant information on the accepted criteria for toxicity.

(*b*) *Conditional acceptance,* allocated if the requirements of the above category of acceptance are not fulfilled to complete satisfaction; in this instance, further investigations required are specified.

(*c*) *Temporary acceptance,* allocated if there are insufficient data to fully establish safety and it is deemed essential that the additional evidence be provided within a stated period of time (usually 3–5 years). If the further data requested do not become available within the stated period, it is possible that the temporary acceptance will be withdrawn by a future Committee.

In some instance *no decision* can be arrived at because the available evidence is inadequate or unsuitable. The fact that a substance is not placed in one of the above categories of acceptance does not imply that there is evidence that it is harmful but merely that, in the opinion of the Committee, its safety has not been established by adequate data. In this connexion it should be noted that lack of essential detail in reports could render impossible a proper evaluation by the Committee and result in "*no decision*" being taken.

It is also conceivable that the Committee might recommend *non-acceptance* if it believed that the available evidence demonstrated a potential health hazard. Such a decision might be accompanied by a recommendation that the use of the particular substance or food should be discontinued.

3.2 Comments on the monographs

Following the pattern established by the Joint FAO/WHO Expert Committee on Food Additives, monographs have been prepared for the

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1968, No. 383.

irradiated foods — wheat, potatoes and onions — considered at this meeting (see Annex 2, p. 17).

Information on the radiation dose required and other technical specifications needed to achieve the stated technological effect were provided by the members invited by FAO and IAEA. The members invited by WHO considered evidence for the wholesomeness of :

(*a*) Wheat or ground wheat products irradiated, using a maximum dose of 75 000 rad, either with gamma-radiation from cobalt-60 or cesium-137 or with electrons from electrical machines, provided that the energy level of the electrons does not exceed 10 MeV.

(*b*) White potatoes irradiated with gamma-radiation from cobalt-60 or cesium-137, using a maximum dose of 15 000 rad.

(*c*) Onions irradiated with gamma-radiation from cobalt-60 or cesium-137, using a maximum dose of 15 000 rad.

In relation to the choice of these doses and technical specifications the following points were made :

(1) The quoted maximum doses were chosen after giving consideration to the over-dosing that might occur in practice in radiation plant operation. The minimum dose used will be the smallest one that gives the required biological effect and, if only for economic reasons, this dose will be the one chosen by the plant operator. The minimum radiation dose required for wheat may vary according to the kinds of insect responsible for infestation in different areas of the world. In the case of potatoes and onions, the radiation dose required may vary for different varieties owing to differences in biological response; climatic differences in various regions of the world may also influence the exact radiation dose required.

(2) The use of electrons has been considered only in some instances and the use of X-rays has not been considered at all, not because they are believed to be entirely unsuitable but because there is insufficient experience of their technological application.

(3) The control of radiation processing within the specifications would be the responsibility of the national authority in the country of operation. Details of appropriate procedures are described in the 1966 report. The control of international trade for the described irradiated products would be based on certification of consignments, stating the dose given and, if possible, a licence number specific to the particular plant where the food was irradiated.

3.3 Conclusions

With irradiated wheat and potatoes, although no positive evidence of harmfulness has been found, the available data contain certain ambiguities and are sometimes lacking in precise details. Accordingly, it was decided that temporary acceptances be recommended for these two irradiated foods. With respect to irradiated onions, the available evidence was found to be inadequate to make a decision on their acceptance.

Summaries of the relevant biological data and details on the evaluation are given in the monographs (pp. 17-44).

4. FUTURE RESEARCH

Man is continually exposed to small doses of potentially harmful materials, and food is but one factor in the total chemical contamination of his environment. The Committee believes that the potential toxicity of irradiated food should not be viewed in isolation, but as a part of the total problem of ensuring that man is exposed to the least possible risk. In this respect, the Committee recognizes that irradiation could replace the use of certain pesticides which are toxic. In the opinion of the Committee this is a strong reason why further exploration into the possible use of food irradiation should be encouraged and supported.

4.1 Data relating to the wholesomeness of irradiated foods in general

Animal feeding studies on individual irradiated foods, using the technical procedures outlined in the report of the 1964 meeting,[1] as modified in section 2 of this report, should be continued.

The report of the 1964 meeting [1] recommended that the irradiation of food for testing should be performed under the practical conditions of processing and that the food should be in a form closely comparable to that consumed by man. The Committee recognizes that these requirements could lead to certain difficulties if interpreted too strictly. For example, the same food, after irradiation, might be processed in a variety of ways, or stored for varying periods of time before eating. Again, the radiation process itself might not be identical in different radiation plants. Therefore, a degree of extrapolation will usually be necessary in order to relate evidence from wholesomeness tests to the practical situation. These comments are particularly relevant when acceptance of an irradiated food for worldwide consumption is under consideration.

In order to permit such an extrapolation to be made with confidence, the Committee believes that it is necessary to take account of all the available data on the wholesomeness of irradiated food. Irradiation is a method of processing food and, as such, data on the wholesomeness of

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1966, No. 316; *FAO Atomic Energy Series*, 1966, No. 6.

one irradiated food have relevance to other irradiated foods. Such generally applicable data are accumulating from specific studies on individual irradiated foods; however, there is much to be said for trying to obtain such data through studies specially designed for this purpose. Such evidence would give added reliability to the evaluation of food treated by a specific irradiation process.

The type of work required to provide data of such wide applicability would be :

1. Animal feeding studies on irradiated diets specifically designed to be representative of broad classes of foods. Such diets could be synthetic or natural food composites. Consideration could also be given to suitable investigations on extracts or concentrates prepared from irradiated foods. The radiation dose given should be within the range of practical interest. The influence of such variables as dose rate might also be examined.

2. The identification and estimation of the products resulting from irradiation of food, and the modification of the yield of such products due to physical and chemical factors, e.g., alterations in environmental conditions before, during, and after irradiation, variations in dose rate, etc.

When reporting animal feeding studies, it is essential that details concerning the type of radiation, total dose, dose rate and dose distribution should be stated, together with the environmental conditions before, during, and after irradiation.

In addition to the studies recommended above, research into the effects of irradiation on the nutritional value of irradiated foods should be continued. In particular, it is important that the nutritive content of the irradiated product as consumed in practice should be checked; for example, the combined effect of irradiation and subsequent cooking might be important.

Further research into the microbiological aspects of the wholesomeness of irradiated foods is also recommended.

4.2 Technical procedures for assessing safety

Research should be continued on the development of new technical procedures suitable for testing the wholesomeness of irradiated foods. Modifications in immunological responses and alterations in behaviour patterns after the consumption of irradiated food deserve further attention as possible sensitive indices of toxicity.

There is a need to find species other than rodents suitable for use in carcinogenicity studies.

Further work on the methods and significance of testing for mutagenicity is also required.

4.3 Methods applicable to regulatory control over food irradiation

It is obviously essential that national legislation governing the production and sale of irradiated food should be enforceable. Control by means of licensing, label declarations, etc. would be greatly strengthened if methods were available that could be applied to the food itself to determine whether or not it had been irradiated. Research into the development of such methods should be continued.

5. RECOMMENDATIONS

1. WHO, in consultation with IAEA and FAO, should give consideration to the possibility of convening further meetings of experts to assess the wholesomeness of irradiated wheat, potatoes, onions and other foods.

2. FAO, IAEA, and WHO should give consideration to the possibility of convening further meetings of experts to undertake revision and/or further clarification of existing documents pertaining to the technical basis for legislation on irradiated foods.

3. Recognizing the importance of proper planning, execution and co-ordination of investigations on the wholesomeness of irradiated food both at the national and international level, it is recommended that the responsible international agencies undertake :

(*a*) to collect data pertaining to wholesomeness;

(*b*) to provide consultation on programmes for testing wholesomeness;

(*c*) to make available technical advice in the planning of experiments to investigate wholesomeness;

(*d*) to encourage research designed to develop new or more adequate methods for testing the wholesomeness of irradiated food.

4. WHO should consider convening a meeting of experts on the assessment of mutagens. Such a meeting should provide guidance for future research on the possible mutagenic hazards due to certain natural components in food and to the consumption of foods containing food additives as well as those subjected to various processing methods, including irradiation.

Annex 1

MUTAGENIC AND CYTOTOXIC CONSIDERATIONS

The Joint FAO/IAEA/WHO Expert Committee on the Technical Basis for Legislation on Irradiated Food, which met in 1964, stated that the subject of mutagenicity would need further consideration when more data had been accumulated.[1] Meanwhile, a re-assessment of the toxicity of all types of environmental factors — food additives, pesticides, drugs, air and water pollutants — has been taking place. This has followed a realization that the testing procedures formerly used did not include tests for detecting subtle hazards to the population, such as teratogenicity and mutagenicity. Obviously, the risk of mutagenicity is not unique to irradiated foods, but should be considered for all substances and for all processes to which food is subjected, e.g., heating.

Several developments have taken place during the last few years (Schubert, 1969). For example,

(*a*) Numerous *in vitro* experiments, both on single-cell organisms and on mammalian and non-mammalian cells, have proved that irradiated foods, food components, and natural products may possess mutagenic and cytotoxic properties;

(*b*) Certain chemical compounds known to cause mutagenic and cytotoxic manifestations have been identified in irradiated model systems; and

(*c*) Mutagenicity tests are being developed that can be performed *in vivo* in mammals.

Several such tests to detect chemical mutagens are described in a recent monograph (Hollaender et al., 1970). For extrapolation of the findings to man, the most useful mutagenic tests are those that are performed *in vivo* in mammals. These could be incorporated into existing testing procedures within a reasonable time, and appear to be suitable for the detection of important classes of genetic damage. Such tests include :

(*a*) The dominant lethal mutation test (Bateman, 1966; Epstein & Shafner, 1968), which involves the sequential mating of male mice or rats, treated or fed with the test substance, with untreated virgin females. Subsequently, the females are autopsied at appropriate times, and the number of implantations and of early deaths and late deaths of fetuses are recorded;

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1966, No. 316; *FAO Atomic Energy Series*, 1966, No. 6, p. 50.

(*b*) The host-mediated assay (Gabridge, Denunzio & Legator, 1969) in which bacteria or *Neurospora* are implanted in the peritoneal cavity of treated mammals. After a period of incubation, the implanted organisms are removed from the peritoneum and the mutation frequency determined. Direct administration or feeding of the test substance to the host allows the animal to activate or detoxify the potential mutagen before it encounters the implanted organism;

(*c*) Cytogenetic analysis of the chromosomes of the bone marrow and meiotic germ cells (Legator & Jacobson, 1968).

REFERENCES

Bateman, A. J. (1966) *Nature* (*Lond.*), **210**, 245

Epstein, S. S. & Shafner, H. (1968) *Nature* (*Lond.*), **219**, 385

Gabridge, M. G., Denunzio, A. & Legator, M. S. (1969) *Nature* (*Lond.*), **221**, 68; *Science* **163**, 689

Hollaender, A., Freise, E. & Legator, M. S., ed. (1970) *Chemical mutagens — principles and methods for their detection*, New York, Plenum Press (in press)

Legator, M. S. & Jacobson, C. (1968) *Detection of cytogenetic effects of toxic agents in mammalian systems.* In : *Manual on the laboratory diagnosis of diseases caused by toxic agents*, Washington, D. C., American Pathologists

Schubert, J. (1969) *Bull. Wld Hlth Org.*, **41**, 873

Annex 2

MONOGRAPHS

IRRADIATED WHEAT

Purpose of radiation treatment

To control infestation by insects in stored grain and in ground wheat products.

Radiation dose

The dose required is dependent on the insect species to be controlled and on the maximum permissible time interval between the time of irradiation and the death of the infesting insects. For most species the required dose is within the range 10 000–25 000 rad; higher doses may be required for the control of mites and certain moths. For the purposes of evaluation, a maximum dose of 75 000 rad has been assumed. Radiation may be either gamma radiation (cobalt-60 or cesium-137) or electrons of energy levels less than 10 MeV.

Biological Data

1. Nutritional studies

Protein. The protein content of wheat and wheat flour, as measured by total nitrogen, is unaffected by irradiation (Calloway & Thomas, 1961; Bierman et al., 1958; Sosedov & Vakar, 1962; Predel'skij et al., 1958; Miller, 1966; Fifield, Golumbic & Pearson, 1967). The amino acid content of wheat flour proteins is unaffected by irradiation with 74 000 rad (Calloway & Thomas, 1961), and no loss has been observed in the limiting amino acid, lysine, even with a dose of 2.8 Mrad (Metta & Johnson, 1959). Small losses in leucine, isoleucine and methionine have been reported with a much higher dose (5 Mrad) than that proposed for disinfestation (Kennedy, 1965a). Irradiation of wheat bran with 0.5 Mrad and with 5.0 Mrad caused no consistent differences in the amino acid composition of the bran proteins (Moran, Summers & Bayley, 1968).

The protein nutritive value of irradiated wheat and wheat gluten has been studied using a microbiological method. The nutritive value of proteins in whole wheat was unaffected when irradiated with a dose of 200 000 rad, whilst with 1 Mrad and with 5 Mrad there was a small but statistically significant loss. The nutritive value of gluten prepared from wheat and then irradiated was not affected when the dose was 20 000 rad but with 200 000 rad, 1 Mrad and 5 Mrad, losses of 5, 7 and 26% respectively occurred. The decreased nutritive value was shown to be due principally to decreased availability of methionine to the test organism. The fact that a similar decrease was not observed when whole wheat was irradiated suggests that the non-protein constituents of wheat exert a protective effect (Kennedy, 1965a). Tests in rats have shown that the protein efficiency ratio (PER), nitrogen excretion, and digestibility are unaffected when wheat is irradiated with 20 000 rad (Gonzales, 1968). The digestibility and biological value of irradiated wheat gluten has been measured in rats by a modification of the Mitchell balance method; wheat gluten from a commercial source that had been irradiated with 2.79 Mrad whilst suspended in water had a digestibility (99%) and a biological value (42%) similar to those of untreated gluten (Metta & Johnson, 1959). Irradiation of wheat bran with 0.4 or 5.0 Mrad gamma radiation caused an increase in net protein utilization in chickens fed a diet in which bran was the sole protein source; supplementation with essential amino acids improved protein utilization in all groups and the supplemented control diet became equivalent in nutritive value to the supplemented diets containing irradiated bran (Moran, Summers & Bayley, 1968).

Carbohydrate. The maltose values of irradiated wheat are substantially increased by irradiation with doses greater than 0.5 Mrad (Linko & Milner, 1960). This is attributed to depolymerization of polysaccharides. In theory, extensive glycosidic bond rupture in indigestible carbohydrates could improve their metabolizable energy; in practice, very high dose levels are required to achieve this objective and irradiation of wheat bran with doses up to 5 Mrad did not increase its metabolizable energy (Moran, Summers & Bayley, 1968).

B vitamins. The evidence regarding the radiosensitivity of thiamine in wheat and wheat flour is conflicting. No loss of thiamine was detected in whole wheat irradiated with 20 000 rad and 200 000 rad (Kennedy, 1965b), or in flour treated with 70 000 rad (Bierman et al., 1958). Other investigators found no significant loss of thiamine in flour irradiated with 25 000 rad, but some small losses were noted with 70 000 rad and higher doses (Poisson et al., 1967). There is some evidence that the amount of thiamine destroyed by irradiation at dose levels between 70 and 500 Krad is a function of the dose received, and is independent of the moisture content of the flour; with 500 Krad about one-quarter of the thiamine present had been destroyed (Causeret, 1967). In contrast, some reports are available in which considerable losses of thiamine have been noted (Calloway & Thomas, 1961; Egiazarov, 1960). About 30-35% of thiamine in a cereal chicken-mash was destroyed with 5 Mrad of gamma radiation (Coates et al., 1963).

There is no loss of riboflavin in wheat flour irradiated with various doses up to 74 000 rad (Bierman et al., 1958; Egiazarov, 1960; Calloway & Thomas, 1961), or in wheat grain irradiated with 20 000 rad or with 200 000 rad of gamma radiation (Kennedy, 1965b). Even with 5 Mrad no loss was evident in a cereal chicken diet (Coates et al., 1963).

There is no loss of nicotinic acid in wheat grain irradiated with 20 000 rad (Kennedy, 1965b) or in wheat flour irradiated with 37 200 rad (Calloway & Thomas, 1961). Small losses have been detected in flours irradiated with 50 000 rad (Egiazarov, 1960) and with 74 400 rad (Calloway & Thomas, 1961). A loss of 12% has been reported for wheat grain irradiated with 200 000 rad (Kennedy, 1965b), although in a chicken mash no loss was demonstrable even with 5 Mrad (Coates et al., 1963).

There was no significant loss in total vitamin B_6 in wheat irradiated with 20 000 or with 200 000 rad (Kennedy, 1965b). In a cereal chick diet sterilized with 5 Mrad of gamma radiation the loss was 20-30%, depending on whether it was irradiated in air or in a vacuum-pack (Coates et al., 1963).

In wheat, there was no significant loss of biotin when irradiated with 20 000 rad, although a loss of 10% was found with 200 000 rad (Kennedy, 1965b). In a cereal chick diet there was no demonstrable loss even with

5 Mrad (Coates et al., 1963). In wheat, losses of pantothenic acid were evident with 20 000 and with 200 000 rad (Kennedy, 1965b), although they were probably too small to be of nutritional concern. In a chicken diet composed mainly of cereals, no loss was observed even with 5 Mrad; nor could any loss of vitamin B_{12} or folic acid be demonstrated (Coates et al., 1963).

Vitamin E. Although in certain foods vitamin E is very labile under the influence of radiation (Becker et al., 1956; Poling et al., 1955), only a small loss was observed in wholemeal flour prepared from wheat irradiated with 20 000 rad and with 200 000 rad (Hickman et al., 1962; Sreenivasan, 1968). This was confirmed in rat-feeding experiments in which virtually the only source of vitamin E in the diet was wheat irradiated with 10 000 rad; although the tocopherol content of wheat, before irradiation, was only just sufficient to meet the requirement for the vitamin, there were no deficiency symptoms in rats raised through 4 successive generations on the diet (Nair & Brownell, 1964). A cereal chicken diet, sterilized with 5 Mrad of gamma radiation, retained about 90% of its vitamin E if vacuum-packed before irradiation. A loss of 50% occurred in the same diet when irradiated in air (Coates et al., 1963). A specific pathogen-free (SPF) breeding colony was maintained for 4 years on an unsupplemented laboratory chow sterilized with 2.5 Mrad of gamma radiation; the breeding performance of this colony was excellent, providing further evidence of the radiation stability of vitamin E in cereals (Hickman, 1966).

Minerals. Irradiation of wheat bran with 0.5 and with 5.0 Mrad caused an increase in the net phosphorus utilization and absolute retention of phosphorus by the chicken (Moran, Summers & Bayley, 1968). In this context, it is of interest to note that phosphorus in the polar fraction increased in lipids of flours milled from wheats irradiated with high doses of gamma radiation (Chung, Finney & Pomeranz, 1967).

Ascorbic acid. No loss of ascorbic acid was observed in flour irradiated with 75 000–80 000 rad (Bierman et al., 1957).

2. Short-term studies

Mouse. Five successive generations of mice were studied in a reproduction study. The initial generation comprised 60 mice divided into 2 groups, one fed wheat irradiated with 27 900 rad of gamma radiation, the other fed untreated wheat. Each mouse was fed 3 g of wheat daily plus 2 g of gruel supplemented with yeast. After about 3 months reproduction was studied and a further 60 mice selected to form the succeeding generation. This was repeated through 5 generations. After their litters had been weaned, the parent mice were again given the stock diet.

In the 4th generation 2 litters were bred (from each female ?) at intervals of 2½ and 4½ months after feeding irradiated or control wheat. Differences were not observed either in the number of young born or in the development of the young. Gross and histopathological investigations did not reveal any abnormalities that could be related to the effect of the irradiated grain (Vasil'jeva et al., 1960).

Rat. A mixture of wholemeal wheat flour (75%) with various Indian vegetables, minerals, and sesame oil was cooked and irradiated with 20 000 rad and 200 000 rad. After storage for 2 months at 10° C, it was used as the diet for 2 groups of 24 weanling rats. A similar group of 24 rats was fed a control diet. No effects on body weight or feed efficiency were observed during 12–15 weeks on these diets. No gross morphological changes that could be attributed to the feeding of irradiated diets were observed in any organ. No significant changes in liver and blood constituents were observed. Electrophoretic patterns of serum proteins also exhibited no differences. In another experiment, the protein efficiency of these mixtures was compared and found unaltered (Vakil, 1969).

Chicken. Cereal grains irradiated with 19 500 rad of gamma radiation were fed to chickens (Rhode Island x Light Sussex) over a period of 6 months when the birds were aged 12 to 18 months. Mixed cereal grains, comprising 3 parts of wheat, 2 parts of maize, 1 part of barley and 1 part of oats, formed 75% of the diet. Pretest observations enabled pairs of hens to be selected from 112 birds, matched for comparable egg production; the two hens in each pair were randomly allocated to control and irradiated diets until 50 pairs had been chosen. There were no significant differences in body-weight between the hens on the 2 diets throughout the 6-month experimental period. The quantity of irradiated and control food ingested was similar in both groups. Egg production, egg weight, and egg quality were also similar in both groups. Autopsy of hens dying during the test revealed that the deaths were due to conditions normally observed in poultry. At the end of the experiment all surviving birds were submitted to gross and microscopic examination for pathological changes. There were no significant differences between groups with respect to body condition, fat deposition in the liver, or development of the ovary. Other lesions occurred in both groups; the differences were not statistically significant. There were no differences in organ weights, fat, nitrogen, nicotinic acid and riboflavin contents of livers, ash weights of femurs, or packed cell volumes and proportions of plasma proteins in terminal blood samples (Cornwell et al., 1960).

Pigeon. There were no differences in reproductive function in pigeons fed unirradiated grain or grain irradiated with 100 000 rad, as assessed by egg production and incubation time, or the weight gains and other indices of development of the offspring (Potehin, 1967a,b).

Dog. Three groups of 2 male and 2 female beagles were fed for 2 years on diets containing flour irradiated with 0, 37 200 and 74 400 rad of gamma radiation of a mixed energy spectrum (spent fuel-element source). After irradiation, the flour was stored at 24–27° C for 36 weeks, and subsequently at −18° C until required, when it was mixed with water to a dough, baked for 2 hours at 170° C, and incorporated into the diet as 35% of the dietary solids. All dogs gained weight rapidly and tended to become so obese that, during the second year of the experiment, it became necessary to regulate their food intake. Dogs fed irradiated flour gained weight slightly faster and had a higher feed conversion than control dogs. This effect was attributed to the increased availability of the irradiated starch due to degradation to smaller molecules. Attempts to breed were only partially successful owing to whelping difficulties and accidents, attributed to the obesity combined with high birth weights of the pups. There were no abnormal findings when dogs were subjected to haematological examinations at 5-weekly intervals throughout the experiment. On post-mortem examination, the liver weight of one dog in the 37 200-rad group appeared unduly high, but the liver weights of all other dogs were normal. Heart, kidney and testes weights were normal. Spleen weights were extremely variable in all groups, probably as a result of hyperaemia in certain animals due to the method of euthanasia. Histopathological examinations revealed thyroid changes — primarily a subacute to chronic thyroiditis with atrophic acini — which increased with increasing radiation dose although the data were too few to have statistical significance (Reber et al., 1959; Reber et al., 1961). These lesions are a normal occurrence in beagle dogs (Ross, Garner & Moseley, 1961; Mawdesley-Thomas & Jolly, 1967; Tucker, 1962; Ross & Hood, 1963).

3. Long-term studies

Mouse. In a carcinogenicity test approximately 400 Cal A strain and 400 C3H NT strain mice, with approximately equal numbers of males and females, were divided into two groups. The animals were fed for 2 years on a mixture of wheat flour, codfish, beef stew, chicken stew, green beans and peaches, each comprising 16.67% of the diet on a dry weight basis. For the test group, the wheat flour was irradiated with 74 400 rad and all other components of the diet with 5.56 Mrad. In the control group, the foods were not irradiated. The diet was cooked by steaming before feeding. Attempts to develop multiparous colonies were unsuccessful owing to the failure of almost all females (control and test groups) to bear more than one litter. There was no effect on bodyweight or on survival. Among the mice of the Cal A strain, 17 of those fed irradiated food developed tumours compared to 11 of those in the control group; all the mice developed lymphosarcomas and 1 mouse in each group was also observed to have pulmonary adenomatosis. Among mice of the C3H NT strain only 3 tumours were observed; one mouse in the irradiated diet group and one in the control group had pulmonary adenomatosis, and one control mouse had parathyroid hyperplasia (Calandra & Kay, 1961).

In a multigeneration test, mice were fed a diet containing 75% of wheat which was either untreated or irradiated with 20 000 rad or with 200 000 rad. Each mouse was mated twice in each generation and data from the first and fourth generations indicated that reproductive capacity was unimpaired (Sreenivasan, 1968).

Rat. Four successive generations of rats were fed diets containing 70% of wheat that was either untreated or irradiated with 10 000 rad of gamma radiation (cobalt-60). Wheat fed to the first 3 generations was stored at room temperature for 1–3 months before feeding. Wheat fed to the fourth generation was stored for 6 months at 80–90° F (27–32° C) in order to test the effects of adverse storage conditions. In each generation, each group comprised 12 male and 12 female rats fed on the test or control wheat diet for 18 months. The initial generation was mated when 12 weeks old to produce the second generation, and again following the weaning of the first litters; second litters were discarded. Successive generations were bred in a similar manner, except that the fourth generation was bred once only. Reproductive performance was assessed in terms of fertility, number of pups born per litter, number of pups surviving the first day postpartum and surviving to weaning, and weaning weight. There was no demonstrable effect except in the second generation, when male fertility was reduced and infant mortality was high in both the first and second litters. In the first litters the weaning weights were low. However, both test and control groups were affected, the control group being slightly more affected than the group fed irradiated wheat. Differences between groups with respect to body-weight were apparent in all generations except the parent generation, but were not consistent. For example, second generation males fed control wheat were heavier than those fed irradiated wheat over a number of weeks during the period of rapid weight gain. However, in the third generation the situation was reversed, rats fed irradiated wheat being heavier. Overall, there was no indication that the consumption of irradiated wheat affects body-weight gain. Detailed histopathological examinations were reported for only 27 rats, which died or were sacrificed during the course of the experiment. No consistent abnormalities attributable to the consumption of irradiated food are reported (Brownell, Horne & Kretlow, 1962).

Three groups of 30 male and 30 female rats in 4 successive generations were fed a diet containing 75% of wheat that was either untreated or irradiated with 20 000 rad or with 200 000 rad of gamma radiation (cobalt-60). The parent generation was mated when 12 weeks old and sufficient offspring were selected at weaning to form the subsequent generation. This procedure was repeated through 4 generations. The second generation was remated when the rats were 23 and 52 weeks old, the offspring being discarded at weaning. There were no significant differences between groups with respect to fertility index, number of pups born, litter size, sex ratio, survival at 3, 7, 10, 14, 17 and 24 days after birth, and body-weight at days 1, 3, 7, 10, 14, 17, 12 and 24. Morphological abnormalities were not observed in any of the pups. The incidence of stillbirths varied in different generations but appears to have been consistent with the random variations that would be expected in the rat (Hickman, McLean & Ley, 1964). The second generation was sacrificed at 1 year for histopathological studies; the other 3 generations were studied until at least 2 years old. There were no consistent differences between groups in body weight, food utilization or survival (Hickman, Greenwood et al., 1964). A total of 1238 animals were autopsied, of which 819 had been maintained on diet for more than 1 year and 247 had been maintained for 2 years or more at the time of sacrifice; a few rats in each group survived 3 years or more before they were killed for histopathological examination. Comprehensive tabulation and statistical appraisal showed that the lesions observed were not related to radiation treatment of the wheat. In particular, the incidence of neoplasms was remarkably similar in the test and control groups; in rats aged 6-12 months, 12-18 months and 18-24 months the proportions bearing tumours were, respectively, 2%, 5% and 25% of the control population, 7%, 0% and 21% of the 20 000-rad group and 2%, 4% and 15% of the group fed wheat irradiated with 200 000 rad. Thus, there is no evidence that irradiated wheat is tumorigenic, nor is there evidence that the consumption of irradiated wheat may influence the age at which tumours become apparent (Greenwood & Jeffries, 1963).

Three groups of rats were maintained for a lifespan and through 5 successive generations on diets containing 75% of wheat that was either untreated or irradiated with 20 000 rad or with 200 000 rad. In each generation, 24 males and 24 females were kept for longevity studies and no differences in survival were observed. Erythrocyte and leucocyte counts were made on 12 animals of both sexes at 3 months of age and were similar in all groups. There was no effect on growth or feed efficiency, nor on intestinal flora as assessed by the numbers, morphology and colony characteristics of faecal *E.coli*. Six males and 12 females of each group were mated. Reproduction in all four generations, as assessed by fertility, litter size, sex ratio, survival to weaning, and birth and weaning weights, was unaffected. Rats of each generation were sacrificed after feeding for 18 months on the test diets; gross and microscopic examination of the heart, lungs, kidney, liver, adrenals and testes showed no abnormalities (Sreenivasan, 1968).

Flour irradiated with 37 200 rad and with 74 000 rad of gamma radiation was fed as 35% of the total dietary solids to groups of 25 male and 25 female rats over a period of 2 years and through 3 generations of offspring. The irradiated flour was stored at room temperature for a minimum of 3 months before incorporation into the ration (control flour stored at 0° C) and, to simulate baking, it was mixed to a dough and cooked at 375° F (190° C) for 120 minutes before feeding. Growth and food intakes in the test and control groups were similar over a 12-week period immediately following weaning in the parent, F_1, and F_2 generations. After the test diets had been fed for 100 days all females were mated to produce the first litters, which were discarded at weaning. The females were remated after an interval of 2-3 weeks to raise second litters, from which groups of 25 males and 25 females were selected at weaning to form the succeeding generation. There were no significant differences between the groups fed irradiated flour and the control group with respect to fertility, number of pups per litter, number of stillbirths, survival to weaning, and weaning weights. A decrease in the breeding performance observed in the F_1 and F_2 generations affected the test and control groups equally. There was no evidence that irradiation of flour causes any reduction in lifespan. Haematological examinations in all generations and throughout the lifespan of the parent generation failed to reveal abnormalities. Gross and microscopic examinations did not indicate any pathological changes that could be related to the ingestion of irradiated flour (Tinsley, Bone & Bubl, 1965). Independent examinations of histological material confirmed that there was no increase in tumour incidence. A significant increase in glomerulotubular lesions in the kidneys of rats fed irradiated flour was reported, but such changes commonly occur in older rats. Dilated sinusoids in the adrenals were also observed more frequently in rats fed irradiated flour; these changes also occur commonly in older rats. Several rats fed irradiated flour had lesions of the intestine, but no control rats showed similar lesions; this effect is inexplicable but is probably fortuitous (Ross & Moseley, 1964). The method of presentation of the data does not permit a comparison of the incidence of these lesions at different ages; consequently, although they may be associated with aging and are probably unrelated to the consumption of irradiated flour, no conclusions can be drawn as to their significance.

Experiments were conducted over a period of 1½ years on 3 generations of rats fed 10 g of wheat daily. In each generation, the animals were divided into two groups of 20 rats, one fed untreated wheat and the other wheat irradiated with 27 900 rad. The first generation was kept under observation for 15 months, the second for 11 months, and the third for 6 months; a fourth generation was bred only to acquire data on early development. The average number of rats born in all 3 generations was comparable in the test and control groups. Growth was unaffected, no effects were observed on blood picture, blood enzymes (catalases and peroxidases), or prothrombin times, and there were no gross or histopathological changes, (Vasil'jeva et al., 1960).

4. Observations in man

Two controlled series of human feeding studies have been reported. In each, foods that had been irradiated with mixed-spectrum gamma radiation from spent fuel elements were fed under carefully controlled conditions to young adult male volunteers during a 15-day period. Each volunteer was also given a control diet during a similar period so that each subject served as his own control. Comprehensive clinical examinations were made on each subject before, during, and after feeding irradiated foods.

In the first investigation, the subjects were fed diets in successive periods, each separated by an interval of several months, in which 35%, 60%, 80% and 100% of the calories were supplied by irradiated foods, including bread and other cereal products, irradiated with 2.79 Mrad and then stored in a frozen state until required. In addition to the clinical examinations, metabolic balance studies were conducted during the first three periods; each subject had clinical examinations at intervals over at least six months following exposure to irradiated food. These studies failed to demonstrate any deleterious effects on general health or damage to the blood-forming organs, liver or kidneys that could be attributed to the ingestion of the irradiated foods (Levy et al., 1957).

In a second series of studies, diets were fed in which approximately 80% of the calories were derived from irradiated foods. Flour, irradiated with 75 000 rad or with 80 000 rad and subsequently stored at room temperature for approximately 3 months was used to prepare various cereal products, including bread and confectionery items. Two studies were conducted, the first on 10 men and the second on 9 men; 6 of the men participated in both studies. There was no evidence of clinical abnormalities that could not be related to the ingestion of irradiated food (Bierman et al., 1958).

5. Other feeding studies

Rat. Three groups of 20 male and 20 female rats were fed for 12 weeks on diets containing bread that was either untreated or irradiated with 25 000 rad or with 50 000 rad; the bread comprised 80% of the diets on a dry weight basis. There was no demonstrable effect on growth or on any of 5 blood enzyme systems examined : erythrocyte transketolase, plasma transaminase, erythrocyte glycolysis, erythrocyte respiration in the presence of methylene blue, and erythrocyte glutathione reductase (Brin, Ostashever & Kalinsky, 1961).

Three groups of 10 male weanling rats were fed a diet containing canned bread which was either untreated or irradiated with 2.79 Mrad or with 5.58 Mrad of gamma radiation (spent fuel elements). The diets contained 35% of bread (dry weight basis) and were nutritionally adequate. During an 8-week period the weight gains and caloric efficiencies were comparable in the control and test groups (Read, Kraybill & Witt, 1958).

Three groups of 10 male weanling rats were fed diets containing 35% (dry weight basis) of a cereal preparation which was either untreated or irradiated with 2.79 Mrad or with 5.58 Mrad of gamma radiation (spent fuel elements). During an 8-week period rats fed the 5.58-Mrad diet gained significantly more weight than the control rats; rats in the 2.79-Mrad group also gained more weight than control rats, but the difference was not statistically significant. A similar effect was observed in parallel studies in which rats were fed irradiated maize, and it is attributed to increased availability of nutrients due to the degradation of cellulose by the irradiation (Read, Kraybill & Witt, 1958).

Chicken. A chick mash containing 30% of ground wheat, 8.5% of wheat offals, 30% of ground maize, and proteins, mineral and vitamin supplements was sterilized with 5 Mrad of gamma radiation (cobalt-60) and used to rear a group of 50 germ-free chicks from hatching until 4 weeks old. Another group of 50 germ-free chicks was fed a similar diet but with the addition of penicillin. For comparison purposes, 4 groups of 50 ordinary chicks were reared under comparable conditions, one on a radiation-sterilized (5 Mrad) diet, one on an unsterilized diet, and the other two on the same diets with penicillin supplements. There were no significant differences between the weights at 4 weeks of age of ordinary chicks given irradiated diets and those given unsterilized diets, with or without penicillin; the germ-free chicks grew significantly better than the ordinary chicks (Coates et al., 1963).

Chickens (17 males and 41 females) were raised and maintained for 13 months on a wet mash diet which was sterilized with 2.79 Mrad of gamma radiation. Toxicity was assessed by comparing these birds with 16 male and 41 female birds fed unirradiated mash. The dietary ingredients were mainly of cereal origin, including wheat middlings and gluten; the proportions were varied throughout the study to allow for the different nutritional requirements of chicks, growing birds and laying pullets. Hens fed the irradiated diet had a slightly lower growth rate than the controls. A decline in egg production and hatchability, accompanied by increased embryo mortality was observed after 6-9 months and appeared to affect birds fed irradiated mash to a greater extent than control birds. However, these effects were reversed by the use of a new source of cod liver oil, and were attributed to vitamin deficiency. There were no differences in the blood picture. Mortality was similar in both groups; gross and histopathological examination of the organs did not reveal any lesions attributable to the consumption of the irradiated diet (Burns, Brownell & Eckstein, 1956).

Mouse. Two groups of 10 male and 50 female C57BL mice were fed a diet containing 50% of wheat flour (70% extraction), which was either untreated or irradiated with 5 Mrad of gamma radiation (cobalt-60). The flour was fed within one week of irradiation. Females and males were caged together for mating; when a female became pregnant it was isolated to deliver and raise its litter, after which it was placed with the male again. This procedure was continued until the mice became too old to reproduce. They were then caged individually until death. The offspring were raised to weaning and sacrificed for chromosomal analysis, examination of the testicles, and blood picture determinations. Cytogenic examinations of the developing spermatogonia in 30 mice of each group revealed that cytogenetic abnormalities were significantly more frequent in the group fed irradiated flour than in the control group. Red cell counts and total and differential white cell counts in the offspring were unchanged. There was no significant effect on fecundity; none of the mice was sterile. In both the test and the control groups, a large number of litters were born in which none of the offspring was viable; the incidence of litters so affected was significantly higher in the group fed irradiated flour. In both groups, there was approximately the same number of young per litter at birth and there was a high death rate between birth and weaning; on the average, the losses during this period were about 35% higher in the test group than in the controls. The lifespan of mice fed irradiated flour was slightly shorter than in the control mice. The overall incidence of tumours was slightly higher in the group fed irradiated flour, but the difference was not statistically significant. However, although tumours of several types were observed in the control animals, nearly all the tumours in the test group were mammary adenomas. Since this type of tumour also occurred in the control mice, the significance of this observation is doubtful. Gross and histopathological examinations of other organs did not reveal any consistent abnormalities (Bugyaki et al., 1968).

Comment

The data reviewed in sections 1–4 are those that primarily influenced the Committee in its evaluation of the safety for consumption of irradiated wheat. However, the Committee took cognizance of certain disturbing effects (reviewed in section 5) in mice fed wheat irradiated with 5 Mrad and consideration of these effects is one important reason for the requirement that certain further work should be undertaken to confirm that similar effects on reproduction are not demonstrable at the dose levels of practical importance.

In general, the evidence concerning nutritive adequacy of irradiated wheat and ground wheat products does not give cause for concern. There is indirect evidence that vitamin D may be destroyed, but it is not clear to what extent this may occur at the dose levels required for disinfestation. However, losses of vitamin D in wheat are considered to be of only minimal importance. The data on thiamine losses are conflicting, but the weight of the evidence suggests that only small losses are likely to occur at the dose levels that are of practical concern. However, the Committee noted a lack of information on the vitamin content of bread prepared from irradiated wheat.

The studies on man reported in section 4 were considered to be of too short duration to add significantly to the evidence on wholesomeness. However, they do give reassurance, since no deleterious effect, by the criteria used, was observed in man after ingesting irradiated food for these short periods.

Evaluation

Extensive studies in animals fed wheat or ground wheat products irradiated with radiation doses of up to 200 000 rad have revealed no evidence that irradiated wheat is toxic or carcinogenic, but some of the studies did yield ambiguous results in certain respects. On this basis a temporary acceptance of wheat and wheat products irradiated with doses up to 75 000 rad is recommended. The further studies specified below should be undertaken and completed within 5 years. Further long-term feeding studies are also desirable.

Further work required before 30 April 1974

1. Additional studies in mice to demonstrate the absence of adverse effect upon reproductive functions when fed flour irradiated with 75 000 rad.

2. An assessment of the nutrient content of bread prepared from irradiated wheat utilizing flours stored for varying periods (up to 2 years) subsequent to irradiation before baking into bread.

REFERENCES

Becker, R. R., Kung, H.-C., Barr, N. F., Pearson, C. S. & King, C. G. (1956) *Food Technol., Champaign*, **10**, 61

Bierman, E. L., Plough, I. C., Sellars, J. H., McGary, V. E., Nevels, E. M., Baker, E. M., Harding, R. S., Richmond, J. & Bowman, B. O. (1958) *US Army Medical Nutrition Laboratory Report* No. 224

Brin, M., Ostashever, A. S. & Kalinsky, H. (1961) *Toxicol. appl. Pharmacol.*, **3**, 606

Brownell, L. E., Horne, T. & Kretlow, W. J. (1962) Petition to US Food and Drug Administration, published by USAEC Division of Technical Information Service Extension, Oak Ridge, Tennessee

Bugyaki, L., Deschreider, A. R., Moutschen, J., Moutschen-Dahmen, M., Thijs, A. & Lafontaine, A. (1968) *Atompraxis*, **14**, No. 3, p. 1

Burns, C. H., Brownell, L. E. & Eckstein, H. C. (1956) *Fed. Proc.*, **15**, 910

Calandra, J. C. & Kay, J. H. (1961) Unpublished report submitted by the US Army

Calloway, D. & Thomas, M. (1961) Unpublished report submitted by the US Army

Causeret (1967) In : *Progrès en vitaminologie. Travaux de la Commission de vitaminologie du Centre national de coordination des études et recherches sur la nutrition et l'alimentation, 3e série*, 1965-1966; *Ann. Nutr.* (*Paris*), **21**, Mémoires originaux, p. 2

Chung, O., Finney, K. F. & Pomeranz, Y. (1967) *J. Food Sci.*, **32**, 315

Coates, M. E., Fuller, E. R., Harrison, G. F., Lev, M. & Suffolk, S. F. (1963) *Brit. J. Nutr.*, **17**, 141

Cornwell, P. B., Greenwood, T., Crook, L. J. & Burson, D. M. (1960) Unpublished report submitted by the UK Atomic Energy Authority

Egiazarov, G. M. (1960) *Vop. Pitan.*, **19**, No. 4, p. 54

Fifield, C. C., Golumbic, C. & Pearson, J. L. (1967) *Cereal Sci. Today*, **12**, 253 & 261

Gonzales, D. A. F. (1968) *An. Bromat.*, **20**, 149

Greenwood, T. & Jeffries, D. J. (1963) Unpublished report submitted by the UK Atomic Energy Authority

Hickman, J. R. (1966) *Food Irradiation : Proceedings of a Symposium, Karlsruhe, 6-10 June 1966*, Vienna, IAEA

Hickman, J. R., Greenwood, T., Bull, J. O., & Ley, F. J. (1964) *Food Cosmet. Toxicol.* **2**, 175

Hickman, J. R., Mc Lean, D. L. A., Law, A. W. & Bostock C. J. M. (1962) *UK Atomic Energy Authority Report*, No. AERE R-4016

Hickman, J. R., McLean, D. L. A. & Ley, F. J. (1964) *Food Cosmet. Toxicol.*, **2**, 15

Kennedy, T. S. (1965a) *J. Sci. Food. Agric.*, **16**, 433

Kennedy, T. S. (1965b) *J. Sci. Food Agric.*, **16**, 81

Levy, L. M., Bernston, L. M., Francis, E., Harding, R. S., Krzywicki, H. J., McGary, V. E., Nuss, J., Sellars, J. H. & Shipman, M. E. (1957) *US Army Medical Nutrition Laboratory Report*, No. 203

Linko, P. & Milner M. (1960) *Cereal Chem.*, **37**, 223

Mawdesley-Thomas, L. E. & Jolly, D. W. (1967) *Vet. Rec.*, **80**, 553

Metta, V. C. & Johnson, B. C. (1959) *Agric. Food Chem.*, **7**, 131

Miller, H. (1966) *Cereal News*, **11**, No. 2, p. 5

Moran, E. T., Summers, J. D. & Bayley, H. S. (1968) *Cereal Chem.*, **45**, 469

Nair, K. K. & Brownell, L. E. (1964) *Radiation Preservation of Foods : Proceedings of an International Conference, Boston*, Washington, D. C., National Academy of Science Publication No. 1273

Poisson, J. M., Jemmali, M., Cahagnier, B. & Leclerc, J. (1967) *Food Irrad. quart. int. Newslet.*, **8**, No. 1-2, p. 2

Poling, C. E., Warner, W. D., Humburg, F. R., Reber, E. F., Urbain, W. M. & Rice E. E. (1965) *Food Res.*, **20**, 193

Potehin, E. V. (1967a) *Some problems of the hygienic and tocixological evaluation of grain exposed to gamma radiation for disinsection purposes.* In : *Materialy naučno-praktičeskoj Konferencii po ispol'zovaniju ionizirujuščih izlučenij v narodnom hozjajstve [Documentation of a Scientific and Practical Conference on the Utilization of Ionizing Radiation in the National Economy]*, Tula, Priokskoe knižnoe isdatel'stvo, p. 207

Potehin, E. V. (1967b) *Vop. gig. Pitan., Učenye zapiski*, p. 44

Predel'skij, A. A., Rodionova, L. Z., Bibergil', A. V. & Rumjancev, P. D. (1958) *Tr. vsesoyuz. naučno-issled. Inst. Zerna*, 3, No. 5, p. 28

Read, M. S., Kraybill, H. F. & Witt, N. F. (1958) *J. Nutr.*, **65**, 39

Reber, E. F., Malhotra, O. P., Kreier, J. P., Norton, H. W. & Beamer, P. D. (1959) *Toxicol. appl. Pharmacol.*, **1**, 55

Reber, E. F., Malhotra, O. P., Simon, J., Kreier, J. P., Beamer, P. D. & Norton, H. W. (1961) *Toxicol. appl. Pharmacol.*, **3**, 568

Ross, M. A., Garner, F. M. & Moseley, M. (1961) Unpublished report submitted by the US Army

Ross, M. A. & Hood, E. (1963) Unpublished report submitted by the US Army

Ross, M. A. & Moseley, M. (1964) Unpublished report submitted by the US Army

Sosedov, N. I. & Vakar, A. B. (1962) *Biohimičneskie osnovy tehnologii piščevyh proizvodstv [Biochemical bases of the technology of food production processes].* In : *Trudy v mezdunarodnogo biohimičeskogo kongressa — Symposium VIII [Proceedings of an International Biochemical Congress — Symposium VIII]*, Moscow, p. 150

Sreenivasan, A. (1968) Unpublished report from Bhabha Atomic Research Centre, India

Tinsley, I. J., Bone, J. F. & Bubl, E. C. (1965) *Toxicol. appl. Pharmacol.*, **7**, 71

Tucker, W. E. (1962) *Amer. J. clin. Path.*, **38**, 70

Vakil, U. K. (1969) In : *Proceedings of a seminar, The Bhabha Atomic Research Centre, Trombay, Bombay, India*, p. 40

Vasil'jeva, Y. N., Okuneva, L. A. & Kukel, Y. P. (1960) *Vop. Pitan.*, **19**, No. 5, p. 59

IRRADIATED POTATOES

Purpose of radiation treatment

To control sprouting in white potatoes during storage.

Radiation dose and type

The dose required varies slightly with different varieties and depends upon the storage period and temperature. For many varieties the dose needed to inhibit sprouting is 7 500–10 000 rad. For the purposes of evaluation the Committee assumed a maximum dose of 15 000 rad of gamma radiation (cobalt-60 or cesium-137).

Biological Data

1. Nutritional studies

Ascorbic acid. Irradiation may cause some slight loss of vitamin C during and immediatly following irradiation (Sal'kova, 1957; Schreiber & Highlands, 1958). The loss is proportional to the radiation dose (Sal'kova, 1957; Groupes de recherche. . ., 1958). With 10 000 rad some workers have observed losses of up to 15% in ascorbic acid (Sal'kova, 1957; Panalaks & Pelletier, 1960), others have observed no losses (Ogata, Iwata & Chachin, 1959). Most of the initial loss of ascorbic acid caused by irradiation can be restored by boiling (Groupes de recherche. . ., 1958). The main cause of loss of ascorbic acid in potatoes is storage; after prolonged storage of 4-9 months there is no difference in ascorbic acid content between unirradiated and irradiated potatoes (Sal'kova, 1957; Panalaks & Pelletier, 1960).

Carbohydrates. The immediate effect of irradiation is an increase in reducing sugars and sucrose (Cloutier et al., 1959; Rubin & Metlitsky, 1958). The behaviour of the sugar content during subsequent storage depends upon temperature; low-temperature storage (40° F; 4° C) results in restoration of the initial condition so that after 4½ months the sugar content of irradiated potatoes is comparable to that of the control potatoes. At higher storage temperatures irradiated potatoes continue to have a higher sugar content; the starch content is correspondingly lower (Cloutier et al., 1959; Burton & Hannan, 1957). It has been suggested that the formation of deoxyglucose from starch by the action of irradiation (Guilbot, 1969) might be used to detect whether or not potatoes have been irradiated with doses in the 10–15 Krad range.

Protein. Irradiation with doses up to 20 000 rad does not significantly alter the amino acid composition of potato proteins (Groupes de recherche. . ., 1958; Fujimaki, Tajima & Matsumoto, 1968). Shortly after irradiation the concentration of certain free amino acids may alter significantly; the concentrations of proline, aspartic acid and other aliphatic amino acids increase while those of the basic amino acids and glutamic acid decrease. However, after 105 days' storage there were no differences in the content of free amino acids between control and irradiated potatoes (Jaarma, 1966; Fujimaki, Tajima & Matsumoto, 1968).

2. Short-term studies

Rat. Two groups of 10 rats were fed 10 g daily of raw, unpeeled potatoes, which were either untreated or irradiated with 9300 rad of gamma radiation (cobalt-60). The dose rate used was exceptionally low; it took 45–50 days to give a 9300 rad dose. Ingestion of irradiated potatoes had no demonstrable effect on growth, blood picture, blood and urine analysis, or on the reproductive performance of the animals (Okuneva, 1958).

Groups of 14 male and 14 female weanling rats were fed during a period of 40 days on diets containing 53% of raw potatoes, which were either untreated or irradiated with 200 000 rad of gamma radiation (cobalt-60). The high potato content of the diet made it unsuitable for rats but the physiological disturbances that resulted were rectified by the addition of hay and straw dust to the diet. Growth was comparable in both groups. Four males and 10 females from each group were mated; the number of offspring per litter was normal. During the lactation period the potato diet was replaced by a diet of higher protein content. Subsequently, the potato diet was fed again during the production of second litters, which were also comparable in both groups with respect to litter size. Careful histological examination of the testes from 7 males per group to assess the activity of the germinal epithelium revealed no differences between the groups. Haemoglobin and haematocrit values were similar in both groups (Jaarma & Henricson, 1964).

In an incompleted long-term experiment, 2 groups of 30 male and 30 female rats were fed a diet containing 72% of dried potatoes, which were either untreated or irradiated with 10 000 rad of electrons. After 1 year there were no differences between the groups with respect to weight gain and protein efficiency (Lang & Bässler, 1966).

Groups of 10 rats were fed during 43 days on a diet containing 75% of dehydrated potatoes prepared from either untreated potatoes or potatoes irradiated with 8000 rad. Although there was a small increase in the digestibility of dry matter and nitrogen-free extracts, the treatment decreased the total digestive nutrients (TDN) and net energy per kg of dry substance (Varela & Moreira-Varela, 1966).

Dog. Six dogs received 200–300 g of cleaned and boiled potatoes daily during a period of 8 months; potatoes fed to 3 of the dogs had been irradiated with 46 500 rad. All dogs fed irradiated potatoes thrived well and their body weights were comparable to those of control dogs. Red and white cell counts, reticulocyte count, blood enzyme (catalase and peroxidase) levels, glucose tolerance curves, prothrombin times, serum cholinesterase, serum albumin, and urine analysis were normal. Post-mortem examination showed liver-fat and lipid-phosphorus to be present in normal amounts. No abnormalities were found on microscopic or macroscopic examination of heart, lungs, liver, kidneys, brain, spinal cord, spleen, pancreas, stomach, intestine, urinary bladder, or gonads (Okuneva, 1958).

Three groups of 2 male and 2 female beagles were fed during 101 weeks on diets in which potatoes formed 35% of the dry matter content. The groups were fed diets containing untreated potatoes, potatoes irradiated with 7000 rad, and potatoes irradiated with 14 000 rad of gamma radiation, respectively. The whole potatoes, including peelings, were cooked and mashed for incorporation into the ration. Body weights were comparable in dogs fed control and irradiated potatoes, but the diet was suboptimal for growth and no dogs attained the normal adult weight. Attempts to mate the animals were unsuccessful, both in the control group and in the groups fed on irradiated potatoes. X-ray examinations of long bones, and haematological examinations performed at regular intervals revealed no differences between the groups (McCay & Rumsey, 1961). Histological specimens from the dogs used in this study were examined by independent workers who concluded that the lesions observed, which were few and generally of a mild nature, were probably unrelated to the ingestion of irradiated potatoes (Ross, Tucker & Moseley, 1962).

Pig. Two groups of 5 male pigs and 1 female pig were fed *ad libitum* on a feed-concentrate with the addition of potatoes, cooked by steaming. One group received untreated potatoes; the other received potatoes irradiated with 10 000 rad of gamma radiation (cobalt-60). Initially the pigs were 11 weeks old, and they were maintained on this ration for 13 weeks. Growth and feed conversion were comparable in both groups. Blood samples taken at 6-weekly intervals revealed no effect on haemoglobin, blood urea, non-protein nitrogen, PCV, plasma specific gravity, or plasma cholesterol. The bacon obtained from pigs in both groups was of similar quality (Horne & Hickman, 1959).

One male and 3 female pigs, aged 3–4 years, were fed for 4 months on 4 kg of potatoes daily, which were irradiated with 14 000–15 000 rad of gamma radiation (cobalt-60); 1 male and 1 female were fed untreated potatoes. In addition, both groups received 2 kg daily of feed-concentrate. The pigs were bred, but the number of litters born (2 in the irradiated diet group and 1 in the control group) was too small to permit any conclusions. The general health was satisfactory. When the animals were slaughtered, those lesions that were observed occurred to a similar degree in both groups. Biochemical investigations on back fat and liver revealed no consistent differences between the two groups (Jaarma & Henricson, 1964).

In another experiment, 2 groups of 2 male and 4 female piglets were raised to maturity (about 6 months old) on a ration of concentrates supplemented with 0.5–3.5 kg of potatoes daily, depending on the age and energy requirements of the animals. One group received untreated potatoes; the other was fed potatoes irradiated with 14 000–15 000 rad of gamma radiation (cobalt-60). There was no effect on fertility, number of offspring born, or the mean weight of offspring. The piglets were healthy and grew normally; two offspring from each group were fed control or irradiated potatoes until they reached 90 kg live-weight without adverse effects on growth or haemoglobin (Jaarma & Bengtsson, 1966).

Five pigs fattened from 25 kg to 90 kg live-weight on irradiated potatoes appeared to have a higher haemoglobin content than pigs fed unirradiated potatoes (Jaarma & Henricson, 1964). In a second experiment, therefore, larger groups consisting of 7 male and 8 female pigs were fattened on a ration of feed-concentrates with 0.5–4 kg of potatoes per pig per day, according to age. One group received potatoes irradiated with 14 000–15 000 rad; the other was fed untreated potatoes. Haemoglobin values were initially comparable in both groups. Although haemoglobin values appeared to increase at a faster rate in certain pigs fed irradiated potatoes, the effect was not consistent throughout the group. There was no significant effect on growth or on the white and red cell counts when measured at 2-week intervals. Plasma glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase, and ornithine-carbamoyl transferase activities, measured at intervals, were similar in both groups. Gross and histopathological examinations of various organs revealed no lesions attributable to the ingestion of irradiated potatoes. The quality of the bacon and the thickness of the back fat were similar in both groups (Jaarma & Bengtsson, 1966).

3. Long-term studies

Rat. In a long-term study extending over 13–14 months, 2 groups of 20 rats were fed 10 g of unpeeled raw potatoes daily; one group was fed untreated potatoes and the other was fed potatoes irradiated with 46 500 rad. All rats thrived normally. Mortality was comparable in the two groups. Growth, red and white cell counts, haemoglobin, reticulocyte count, blood enzymes (peroxidase and catalase), glucose tolerance, prothrombin times, serum cholinesterase, serum albumin, and urine analysis were all normal. Reproductive performance, as assessed by litter size, birth weight and the morphological development of the offspring, was comparable in the test and control groups. Twelve offspring in each group were selected at weaning and studied for 1 year while on a diet of irradiated potatoes; no abnormalities were noted. On post-mortem examination no significant changes were observed in the organs (Okuneva, 1958).

Potatoes irradiated with doses of 13 500–20 000 rad and 27 000–40 000 rad of gamma radiation were fed to rats as 35% of the diet (dry weight) for 2 years and through 4 successive generations. The skins and eyes of the potatoes as well as the whites were included in the diet; the potatoes were cooked by steaming before feeding. Initially 25 male and 25 female rats were fed on potatoes treated at each irradiation level. Another group was fed a similar diet containing untreated potatoes. Corresponding animals in each group were litter-mates. Twenty females in each group were bred; from the second litters 25 males and 25 females per group were selected to form the next generation. This procedure was repeated until 4 generations had been studied. Females fed potatoes treated at 13 500–20 000 rad (low-dose group) had the lowest fertility and the highest resorption rate; however, since there were no differences in these respects between the control group and the group fed potatoes irradiated with 27 000–40 000 rad (high-dose group), it was concluded that the differences observed in the low-dose group were anomalous. There was no effect on litter size or preweaning survival of pups. In the third generation the average weight of pups in the high-dose group was less than that for the other groups; no similar effect was observed in the first or second generations. Growth and food efficiency were comparable in all groups, except that a slight but significant ($P = 0.01$) increase in body weight was noted in first generation females in the low-dose group at 24 weeks and subsequently; however, this effect was not apparent in the second and third generations. Haemotological examinations at 4, 10 and 18 months of age were normal. Although, in the first and second generations the mortality rate of male and female rats fed the irradiated potatoes was consistently slightly higher than that of control rats, the difference were not statistically significant; the higher mortality observed appears to be related to a greater incidence of respiratory disease in rats fed on irradiated potatoes. The ratio of lung to body weight was lower in males in the high-dose group and in females in the low-dose group; lung weights in females of the high-dose group were comparable to lung weights of control females. Relative spleen weights were lower in females in the low-dose group but not in those in the high-dose group. There were no effects on the weights of the heart, kidney, liver or testes. Coronary arteriosclerosis and focal myocarditis were reported more frequently in rats fed irradiated potatoes than in rats fed either untreated potatoes or laboratory chow. Bronchiectasis with associated pneumonitis and abscesses was also more frequent in rats fed irradiated potatoes than in the control groups. Other pathological lesions occurred with approximately equal frequency in all 3 groups. The frequency of malignant tumours was similar in the control and test groups. In the low-dose group a total of 37 benign tumours was recorded compared to 26 in the control group; however, in the high-dose group 22 benign tumours were noted (Burns, Abrams & Brownell, 1960). An independent group of pathologists who examined histological preparations from animals in this study reported slightly different findings : they found that the percentage of animals with benign tumours in the control, low-dose, and high-dose groups was 89, 88, and 88 respectively, while the percentage of animals with malignant tumours was 48, 40 and 30 respectively. A higher incidence of intestinal nephritis was observed in males in the high-dose group than in those in the control and low-level groups, and a higher incidence of major lesions of the testes occurred in rats of both groups fed irradiated potatoes than in control rats (Ross & Hood, 1966).

In another 4-generation study in rats, the effects of feeding diets containing 35% (dry weight basis) of potatoes irradiated at 7 500–8 500 rad or 15 000–17 000 rad of gamma radiation were assessed by comparison with a similar diet containing untreated potatoes. The potatoes, including skins, were cooked by steaming before incorporation into the diet; during the experiment some of the control potatoes sprouted and were inadvertently fed, on at least one occasion, with consequent alkaloid poisoning in the control group; this resulted in a high incidence of stillbirths and a high neonatal mortality. The initial generation comprised 26 male and 26 female rats in each group; these animals were maintained on the diets for 2 years. Subsequent generations comprised 20 males and 20 females selected from the second litter at weaning, except for the fourth generation which was sacrificed at weaning (Kline, von Elbe & Birdsall, 1960). Unfortunately, the report of this experiment shows a number of inconsistencies and certain fundamental errors in the calculation of the results. Therefore, data from this investigation were not considered in evaluating the safety for consumption of irradiated potatoes. However, a group of pathologists who made independent examination of histological material from this investigation reported that the only significant findings were an apparently higher incidence of congestion or oedema of the spleen and, in rats fed potatoes irradiated with 15 000–17 000 rad, occasional minor lesions (granuloma, haemosiderosis, or hyperplasia in the mesenteric lymph nodes); also, significantly more females in this group had minor parenchymal changes in the liver than in the control group (Ross & Moseley, 1962).

Comment

Few of the long-term studies on irradiated potatoes are considered entirely satisfactory. In general, the investigations were not well planned and appear to have been poorly executed. The remaining data were considered too few to permit an assessment of chronic hazard to be made.

Assessment

In spite of the generally unsatisfactory nature of many of the long-term feeding experiments in animals, the Committee did not find any consistent evidence to suggest that irradiated potatoes are harmful. Temporary acceptance of white potatoes irradiated for the purpose of controlling sprouting is recommended, provided that the dose does not exceed 15 000 rad of gamma radiation from cobalt-60 or cesium-137. Further long-term feeding studies are desirable. The further work required is as follows :

Further work required by 30 April 1974

(1) Investigations using adequate numbers of rats and mice under carefully controlled conditions to make certain that there is no effect on reproductive function.

(2) An assessment of carcinogenicity in a second species of animals (e.g., mouse) fed irradiated potatoes in their diet.

REFERENCES

Burns, C. H., Abrams, G. D. & Brownell, L. E. (1960) *Toxicol. appl. Pharmacol.*, **2**, 111

Burton, W. G. & Hannan, R. S. (1957) *J. Sci. Food Agric.*, **12**, 707

Cloutier, J. A. R., Cox, C. E., Manson, J. M., Clay, M. G. & Johnson, L. E. (1959) *Food Res.*, **24**, 659

Fujimaki, M., Tajima, M. & Matsumoto, T. (1968) *Agric. biol. Chem.*, **32**, 1228

Groupes de recherche du laboratoire central des subsistances de la défense nationale (Paris) et de la société conservatome (Lyon) (1958) *Analytical study of potato tubers subjected to nuclear radiation.* In : *Proceedings of the Second United Nations International Conference on the Peaceful Uses of Atomic Energy*, vol. 27, Geneva, United Nations, p. 434

Guilbot, A. (1969) Unpublished report submitted to WHO

Horne, T. & Hickman, J. R. (1959) *Int. J. appl. Radiat.*, **6**, 255

Jaarma, M. & Bengtsson, G. (1966) *Nutr. et Dieta*, **8**, 109

Jaarma, M. (1966) *Acta chem. scand.*, **20**, 323

Jaarma, M. & Henricson, B. (1964) *Acta vet. scand.* , **5**, 238

Kline, B. E., von Elbe, H. & Birdsall, J. J. (1960) Unpublished report submitted by the U.S. Army

Lang, K. & Bässler, K. H. (1966) *Nutritional value of irradiated potatoes.* In : *Food Irradiation — Proceedings of a Symposium, Karlsruhe, 6-10 June 1966*, Vienna, IAEA, p. 167 (abstract)

McCay, C. M. & Rumsey, G. L. (1961) Unpublished report submitted by the U.S. Army

Ogata, K., Iwata, T. & Chachin, K. (1959) *Bull. Inst. chem. Res., Kyoto Univ.*, **37**, No. 5-6

Okuneva, L. A. (1958) *Vop. Pitan.*, **17**, No. 5, p. 49

Panalaks, T. & Pelletier, O. (1960) *Food Res.*, **25**, 33

Ross, M. A. & Hood, E. (1966) *Fed. Proc.*, **25**, 1549

Ross, M. A. & Moseley, M. (1962) Unpublished report submitted by the U.S. Army

Ross, M. A., Tucker, W. E. & Moseley, M. (1962) Unpublished report submitted by the U.S. Army

Rubin, B. A. & Metlitsky, L. V. (1958) *A study of the action of ionizing radiation on the metabolism of potato tubers...* In : *Prooceedings of the Second United Nations International Conference on the Peaceful Uses of Atomic Energy*, vol. 27, Geneva, United Nations, p. 437

Sal'kova, E. G. (1957) *Dokl. Akad. Nauk. SSSR*, **114**, No. 4, p. 757

Schreiber, J. S. & Highlands, M. E. (1958) *Food Res.*, **23**, 464

IRRADIATED ONIONS

Purpose of radiation treatment

To control sprouting in onions.

Radiation dose and type

The dose recommended is usually between 8000 and 10 000 rad. For the purposes of evaluation the Committee assumed a maximum dose of 15 000 rad of gamma radiation from cobalt-60 and cesium-137.

Biological Data

1. Nutritional studies

Ascorbic acid. Irradiation with doses up to 12 000 rad had little effect on the ascorbic acid content of onions when measured at various intervals up to 7½ months after irradiation (Johnston, 1962). There was no conversion of reduced ascorbic acid to the dehydro form (Mathur, 1962).

Carbohydrate. Irradiation with doses up to 14 900 rad caused no consistent effects on the total sugar content and reducing sugars of onions stored at 50°F (10°C) (Johnston, 1962). Other workers have reported an accumulation of non-reducing sugars in irradiated bulbs (Mathur, 1962).

2. Short-term studies

Rat. Two groups of 15 male and 15 female rats were fed diets containing 3% of dehydrated onion (equivalent to 25% of onion on a wet-weight basis). The onions used to prepare dehydrated onion for one group had been irradiated with 20 300 rad of gamma radiation. Weight gains and food intakes were studied over a 12-week period and were normal. Erythrocyte counts and haemoglobin levels were lower in rats fed irradiated onions, although still within the normal limits for the species. No differences were apparent when total leucocyte counts and haematocrit values were compared for rats fed irradiated onions and control rats (Oliver, Hilliard & van Petten, 1966).

Unirradiated onion and onion irradiated with 8000 and 100 000 rad of gamma radiation were incorporated into the diet of rats in amounts equivalent to 10% and 25% wet-weight. The animals were maintained on this diet for 12 months. Each group contained 25 male and 25 female rats. After irradiation the onions were cooked, peeled, dehydrated and ground to be stored until required for feeding. Growth, reproduction (number of litters, litter size, survival to age 25 days, weaning weight) and blood picture were unaffected by the consumption of irradiated onion, even at the higher dose level. Six male and 6 female rats from each group were sacrificed at 34 weeks of age for gross and histopathological examination; no significant deviations from normality were found. Sixteen male and 16 female rats were autopsied at 56 weeks of age. No significant effects were observed upon gross or microscopical examination, and there was no significant effect on the weight of the heart, kidney, spleen or testes (van Petten, Oliver & Hilliard, 1966). Eighteen male and 18 female rats in each group were selected at random from the second litters and maintained to 25 weeks of age on the same diets as their parents. Third generation rats were similarly maintained to 6 weeks of age. In the second generation, haematological examinations at 14 weeks of age showed significantly lower haematocrit values in males fed a diet containing 10% of irradiated onion; total leucocyte counts showed some significant differences between groups, but the differences were inconsistent. However, further haematological examinations of second generation rats at 25 weeks of age and of third generation rats at 6 weeks of age revealed no significant differences. At autopsy there were no abnormalities that could be attributed to the consumption of irradiated onion. The weights of the heart, kidney and spleen were unaffected (van Petten, Hilliard & Oliver, 1966).

Two groups, each of 70 male and 70 female rats, were fed for a period of 90 days on diets containing 35% of onions (dry weight basis), which were either untreated or irradiated with 25 000 rad of gamma radiation. The onions were air-dried and ground to a powder to facilitate storage and incorporation into the diet. A comparable group of rats was fed a diet containing casein, which was isocaloric and isonitrogenous with the diets containing onion. There were no consistent effects on weight gain. No differences between groups were apparent when food intakes, blood picture (haemoglobin, haematocrit, red cell count, total and differential white cell count), blood chemistry (SGPT, urea nitrogen, and bilirubin), urinalysis, and intestinal glutamic oxalacetic transaminase were compared. Organ weights were normal except for increased spleen weights in both groups fed onions. At autopsy, the spleens of rats in both groups fed onions were observed to be congested and pigmentation (probably haemosiderin) with myeloid metaplasia and reticuloendothelial hyperplasia were noted. In addition, pigmentation of the liver and kidney was reported. In the liver, in addition to the pigment-laden macrophages along the sinusoids and around the central veins, leucocytes of other types were present; this was considered to be a mild hepatic leucocytosis. These changes, which affected rats in both groups fed onions, were not observed in animals fed the casein diet. Other lesions that occurred were those frequently observed in rats; animals in all groups, including the casein controls, were affected (Gabriel & Edmonds, 1966).

Dog. During a period of 90 days, groups of 4 male and 4 female beagle dogs, initially 3 months old, were fed diets containing either 10% of untreated dried onion or 10% of onion that had been irradiated with 25 000 rad of gamma radiation (cobalt-60) and dried. For comparison purposes 8 male and 8 female beagles were concurrently maintained on an isocaloric, isonitrogenous diet that contained no onions. Urinalysis at intervals throughout the test and body-weight measurements revealed no differences between groups. Haematological examinations were inconclusive. Dogs in both groups fed onions were anaemic initially and this anaemia became more severe as the test progressed; it appeared that the anaemia progressed slightly more rapidly in dogs fed irradiated onion than in dogs fed non-irradiated onion, so that after 90 days the erythrocyte count and haematocrit values for dogs fed irradiated onion were marginally lower than for dogs fed non-irradiated onion. However, under the conditions of this experiment (i.e., small number of animals, animals anaemic initially) the biological significance of this observation cannot be assessed. At intervals, blood samples were analysed for urea nitrogen, SGPT and bilirubin; differences between the dogs fed onions and those fed diets containing no onions appear to have been present before the test was started, and there were no apparent differences between dogs fed untreated onions and those fed irradiated onions. At autopsy dogs fed onions, whether irradiated or not, had increased spleen weights due to congestion, pigmentation, myeloid metaplasia, and reticuloendothelial hyperplasia. These animals also had pigment deposition in the liver and kidney and mild leucocytosis. The foregoing abnormalities are probably all attributable to haemolysis of the red blood cells caused by abnormally high dietary intakes of onions; similar effects were not observed in the group not fed onions. Other lesions observed were of types commonly observed in dogs, and occurred in equal or greater degree in the control animals (Gabriel & Edmonds, 1966).

Two groups of 2 male and 3 female Basenji X beagle dogs were fed for 18 months on a commercial dog food to which dehydrated onion had been added in a proportion equivalent to 25% wet weight. One group received dehydrate donion prepared from untreated onions, the other received dehydrated onion prepared from onions irradiated with 20 000 rad of gamma radiation. Another group of 1 male and 2 females was kept on the commercial dog food without onion. The dogs remained healthy; growth and food consumption were unaffected. Dogs fed onions, whether irradiated or not, tended to become anaemic; there were no significant differences between groups. No gross changes were apparent at autopsy. Moderate amounts of haemosiderin observed in the livers and spleens would be expected in animals with a degree of haemolytic anaemia. All animals fed onions, whether irradiated or not, had varying degrees of ovarian or testicular degeneration (Hilliard, Oliver & van Petten, 1966).

Pig. Dehydrated onion, in a proportion equivalent to 25% wet weight, was added to the diet of 2 groups of pigs, each consisting of 2 males and 2 females. The pigs were fed from the age of 8 weeks during a 24-week period. One group received dehydrated onions prepared from onions treated with 20 300 rad of gamma radiation; the onions fed to the other group were not irradiated. Two pigs in the irradiated onion group developed lameness; the syndrome was tentativaly diagnosed as either chronic erysipeloid arthritis or osteodystrophy due to a calcium and phosphorus imbalance. Autopsy revealed that this syndrome was present in varying degrees in all pigs, including those in the control group. Decreased weight gains and food intakes were associated with the osteodystrophy. There were no effects on erythrocyte counts or haemoglobin concentrations, but the haematocrit values during weeks 13 to 24 were significantly higher in the pigs fed irradiated onions. During the last half of the test the leucocyte count was significantly higher in the group fed irradiated onions; this effect may have been related to the more severe osteodystrophy observed in 2 pigs in this group (Oliver, Hilliard & van Petten, 1966).

3. Long-term studies

No long-term studies on irradiated onions are available.

4. Observations in man

There have been no controlled feeding studies in man using irradiated onions.

Comment

Because onions contain active constituents that cause haemolysis and anaemia in nearly every species of animal, it is difficult to feed diets with a high content of onions. In those feeding studies that have been performed with irradiated onion, the appearance of toxic symptoms in the control animals has made it almost impossible to decide whether or not toxic effects have occurred due to irradiation. Clearly, adequate toxicological testing of irradiated onions presents a problem. The Committee suggests that a search should be made for more suitable test species that can better tolerate onions or for other suitable test methods. Also, since it is evident that the levels of dietary intake that can be achieved are likely to be quite low, tests on a larger number of animals than usual may be necessary.

Evaluation

The data relating to the wholesomeness of onions irradiated with doses up to 15 000 rad of gamma radiation are not satisfactory for an evaluation. Although the studies reported do not indicate any harmful effect due to irradiation, the results are complicated by the fact that the high levels of onion fed caused toxic symptoms in the control animals as well as in those fed the irradiated diet; furthermore, none of the studies was continued for a sufficiently long period to permit an assessment of possible carcinogenicity.

REFERENCES

Gabriel, K. L. & Edmonds, R. S. (1966) Unpublished data submitted by U.S. Atomic Energy Commission

Hilliard, W. G., Oliver, W. T. & van Petten, G. R. (1966) *Food Cosmet. Toxicol.*, **4**, 577

Johnston, F. B. (1962) Unpublished data submitted by Atomic Energy of Canada Ltd.

Mathur, P. B. (1962) *Food Irrad.*, **3**, No. 1-2, p. A2

Oliver, W. T., Hilliard, W. G. & van Petten, G. R. (1966) *Food Cosmet. Toxicol.*, **4**, 569

van Petten, G. R., Hilliard, W. G. & Oliver, W. T. (1966) *Food Cosmet. Toxicol.*, **4**, 593

van Petten, G. R., Oliver, W. T. & Hilliard, W. G. (1966) *Food Cosmet. Toxicol.*, **4**, 585