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THE TEXT OF AN AGREEMENT FOR COLLABORATION IN AN INTERNATIONAL PROGRAMME ON IRRADIATION OF FRUIT AND FRUIT JUICES

The text of the Agreement between the Agency, the Österreichische Studiengesellschaft für Atomenergie GmbH and the Organisation for Economic Co-operation and Development for Collaboration in an International Programme on Irradiation of Fruit and Fruit Juices, which was signed on 16 September 1964 and entered into force on 1 January 1965, is reproduced in this document for the information of all Members of the Agency.

Until 31 December 1970

Review of agreement after 3 years

AGREEMENT FOR COLLABORATION IN AN INTERNATIONAL PROGRAMME ON IRRADIATION OF FRUIT AND FRUIT JUICES

The Österreichische Studiengesellschaft für Atomenergie GmbH, the Organisation for Economic Co-operation and Development and the International Atomic Energy Agency;

CONSIDERING that the Österreichische Studiengesellschaft für Atomenergie GmbH (hereinafter referred to as the « S.G.A.E. ») has constructed laboratories and offices for its Institute of Biology and Agriculture at the Seibersdorf Reactor Centre; that part of the major equipment is installed and that the remainder will be installed by the end of 1964, and that it is intended to build and complete an irradiation plant and a cooling plant by the beginning of 1966;

CONSIDERING that in the course of studies undertaken within the European Nuclear Energy Agency (hereinafter referred to as « ENEA ») of the Organisation for Economic Co-operation and Development with a view to co-operation in the field of food irradiation, the S.G.A.E. has stated that it is ready to envisage the carrying out of an international programme of research on the irradiation of fruit and fruit juices at the Seibersdorf Reactor Centre, and that a certain number of Member and Associate countries of ENEA have expressed their interest in collaborating in such an international programme;

CONSIDERING that the International Atomic Energy Agency (hereinafter referred to as the « I.A.E.A. ») has expressed its interest in collaborating in such an international programme;

DESIROUS of establishing the conditions under which such an international programme will be carried out, the legal acts relating thereto being performed by the S.G.A.E.;

HAVE AGREED as follows:

Article 1

(a) The S.G.A.E., the interested countries of ENEA, and the I.A.E.A. will collaborate in an international programme at the Seibersdorf Reactor Centre on the basis of the draft programme proposed by the S.G.A.E. and contained in Annex I to this Agreement, and in accordance with the provisions of this Agreement.

(b) The Member and Associate countries of ENEA interested in participating in the programme under the conditions laid down in this Agreement (hereinafter referred to as « Participating Countries ») shall inform ENEA accordingly and ENEA shall notify the S.G.A.E. of the intended participation.

(c) The S.G.A.E. shall make available for carrying out the programme both the installations and equipment defined in Annex II to this Agreement as well as administrative services for the duration of the programme and shall bear all expenses connected therewith.

Article 2

(a) The Participating Countries shall arrange for the secondment of qualified scientists to participate in the programme.

(b) Secondments shall be made in agreement with the S.G.A.E., and seconded staff shall carry out their work under the direction of the Project Leader. All salaries and costs for the seconded staff shall be borne by their parent bodies.

(c) A Member or Associate country of ENEA may, with the agreement of the S.G.A.E. and subject to the approval of the Project Committee, become a Participating Country by making a financial contribution for the use of the programme in place of, or in addition to, arranging for the secondment of qualified scientists.

(d) A Member or Associate country of ENEA may, with the agreement of the S.G.A.E. and subject to the approval of the Project Committee, become a Participating Country by carrying out special research work as part of the programme in place of, or in addition to, arranging for the secondment of qualified scientists.

Article 3

The I.A.E.A. shall extend its support to the programme in agreement with the S.G.A.E., in particular by awarding fellowships to qualified scientists from its Member States for participation in the programme. The I.A.E.A. may also, in agreement with the S.G.A.E., assign experts for a limited period of time to participate in the programme. Recipients of fellowships and experts shall carry out their work under the direction of the Project Leader. The I.A.E.A. may terminate its participation in the programme by giving twelve months' notice to the S.G.A.E. and ENEA.

Article 4

(a) A Project Committee composed of one member designated by each of the Participating Countries, one member designated by ENEA, one member designated by the I.A.E.A. and not more than four members designated by the S.G.A.E, shall:

(i) Approve each year the programme elaborated by the Project Leader, including any special research work connected therewith. To the extent that the programme involves the use of installations, equipment, or administrative services of the kind referred to in Article 1 *(c)* other than those required to support the programme as outlined in Annex I, the assent of the S.G.A.E. shall be required;

(ii) Give advice on the use to be made of any financial contributions received from Participating Countries;

(iii) Give advice on health and safety measures;

(iv) Deal with any other matter brought before it by the Project Leader.

(b) The Project Committee shall designate each year a Chairman and Vice-Chairman and settle its own Rules of Procedure. It will meet at least twice a year and shall be convened by its Chairman.

(c) The Project Committee shall work out rules relating to the distribution and utilisation of the scientific and technical information derived from the carrying out of the programme.

(d) The Project Committee shall keep the Steering Committee of ENEA and the I.A.E.A. informed of the general progress of the work.

Article 5

(a) The Project Leader shall be appointed by the S.G.A.E. after consultation with the Project Committee and shall be responsible for the carrying out of the programme. He shall be assisted by a Technical Adviser appointed in agreement with ENEA and the I.A.E.A.

(b) The Project Leader shall carry out his tasks in consultation with the Project Committee. He shall attend meetings of the Project Committee, and supply all information which is requested of him concerning the carrying out of the programme.

(c) The Project Leader shall submit quarterly reports to the Project Committee on the progress of the programme.

Article 6

The health and safety standards to be applied in carrying out the programme shall be consistent with the Basic Safety Standards for Radiation Protection of the I.A.E.A. and with the Radiation Protection Norms of ENEA.

Article 7

The S.G.A.E. shall indemnify the Participating Countries, the I.A.E.A. and ENEA as well as any person acting on behalf of any one of them and participating or assisting in the programme, in respect of any actions, claims, costs and expenses arising out of damage resulting from the carrying out of the programme: provided that, where such damage results from an act or omission done with intent to cause damage, the S.G.A.E. will not be required to indemnify the Participating Country or the organisation on whose behalf any such person was so acting.

Article 8

(a) This Agreement shall be concluded for a period of six years from 1st January 1965.

(b) No later than three years after the entry into force of this Agreement the Participating Countries, the S.G.A.E., ENEA and the I.A.E.A. shall review the state of progress of the programme and assess the scientific and technical results obtained. In the light of this assessment, they shall determine the conditions under which

the programme may be continued, including the time-table, as well as any consequential revision of this Agreement.

(c) The S.G.A.E. may, in consultation with the Project Committee, enter into agreements for collaboration in furtherance of the programme.

ANNEX I

OUTLINE OF PROGRAMME

INTRODUCTION

The programme is based on the use of fruit and fruit juice as model substances for fundamental research in the acquisition of basic knowledge of broad application in food irradiation. It is concerned mainly with wholesomeness tests and with microbiological investigations.

The wholesomeness studies will comprise simultaneous testing of several different fruits and fruit juices in both long-term and short-term animal feeding programmes. In addition, chemical separation and identification of the compounds formed by irradiation will be carried out, and biological screening by means of test systems such as micro-organisms or animal and plant tissue cultures will be used in the determination of possible toxic constituents. If such constituents can be isolated, toxicity investigations will be carried out on biological test systems and on special research animals.

The microbiology programme will be concerned principally with irradiation sensitivity of yeasts under various conditions. These studies may be useful in the solution of problems connected with yeast activity in general.

Systematic investigations will also be made of the changes in flavour and

appearance caused by irradiation. Finally, in the later stages of the programme, attention will be paid to the practical applications of the results obtained.

The staff requirements will vary during the carrying out of the programme from about 10 to a maximum of 20 scientists with about twice these numbers in auxiliary staff.

I. WHOLESOMENESS TESTS

General

1. When any new food process is introduced it must be borne in mind that the treated food is likely to be consumed by man over the greater part of his life-span. Evidence is therefore required to demonstrate the absence of toxic or carcinogenic risks following consumption of the food.

Long-term animal feeding tests give the basic necessary evidence for the absence of toxic compounds and carcinogens in processed foods. These tests must be carefully designed with each particular application and food in mind, and feeding tests must be adequately supported by biochemical research into the changes occurring in the food on processing and the effect of such changes on metabolism.

The methods for testing the safety of food additives have been outlined in the second and fifth reports of the joint FAO/WHO Expert Committee on Food Additives. When testing the safety of an irradiation process, the basic principles laid down in these reports should be adopted. However, in the testing of irradiated foods, certain problems arise which do not occur in food additive testing. The nature of the potentially toxic material under test is not known and hence injection and painting techniques cannot be applied to demonstrate the absence of toxic factors. Such techniques are, however, valuable links in the fundamental research aimed at an understanding of the biological consequences of food irradiation.

The fact that the nature of a potential toxic compound is not known also makes it difficult to test this compound at a pre-determined safety margin. Therefore the irradiated food itself must be regarded as the « additive » and safety margins can only be derived from the possible intake of the food. For many foods the normal human intake is so large that rations cannot be formulated for animal tests with high safety margins when dosage is related to body weight. Irradiation doses greater than those to be used in practice are of value mainly in assessing the potential hazard of accidental over-irradiation. The biological effects produced by over-irradiated material do not necessarily reflect effects occurring with lower doses

of radiation since radiation-induced products in food may, themselves, be changed with higher doses.

2. The animal tests as described below will be based on the recommendations of the F.A.O., W.H.O. and I.A.E.A.

The animals used for the experiments will be mainly rats and mice. The animals will be bred in the farm attached to the laboratories in order to study the influence of environmental factors before carrying out the feeding tests, and will be housed in air-conditioned rooms, about 3m × 3 m × 2m in dimensions.

The long-term feeding tests for fruit and fruit juices would consist of a combined feeding of a freeze-dried mixture of irradiated fruits (different species and different sorts) plus irradiated fruit juices. In this way a whole group of important foods could be tested simultaneously and possibly cleared.

Studies with mice will be on a single generation and will require about two years. The rat tests will be four-generation studies and will require about five years for completion.

SINGLE-GENERATION TESTS ON MICE

3. It is intended to test the irradiated mixed diet mentioned above on at least two strains of mice. One will have a low spontaneous tumour rate, but will not be tumour resistant: the CBA strain is suitable for this test. The other will carry a fairly high incidence of spontaneous tumours and for this the C3H strain has been considered.

With the CBA mice, investigations will be made over 80 weeks and the possibility of carcinogenic properties of irradiated fruit and fruit juices will be examined. The C3H mice will be used to investigate the presence of any substance in the irradiated food which might change natural tumour development.

MULTI-GENERATION TESTS ON RATS

4. The selection of a suitable strain of rats will need considerable thought. Wistar strain albino rats have mainly been used for this type of experiment in the past, but most colonies are susceptible to respiratory disease. A « hooded » strain, or the use of so-called « pathogen-free » strains, might be preferable. Rats used in the experiments will be obtained from the project's own breeding colony. During the period in which the colony is being developed, information will be gained on the optimal environmental conditions to give minimal variation of biological response.

The actual long-term experiments on rats will not be started until the details of irradiation doses and conditions are known. During the initial stage of the experiments, the characteristics of normal rats in the colony will be studied.

In addition to the multi-generation tests, about 20 or more animals per group will be kept apart from the trials for pathological examination, which will take place at the age of 4 months, 9 months and 14 months.

The animal population for the whole test programme will thus reach its maximum some twelve months from the start, and number about 1,200. Allowing for a certain number as a reserve, a total of some 2,000 rats will be required.

5. The pathological studies will be made on both research animals and on those from the long-term tests, at the end of their life-span. They will cover all the organs as well as the blood cells. Special histo-chemical techniques will be adopted. Statistics on growth and reproduction will also be worked out.

6. Since chemical changes in food might cause toxicity or carcinogenicity, investigations in this field are very important in connection with wholesomeness. These investigations cannot replace animal feeding tests, but on the other hand animal feeding tests can only give clearance for one special food item processed in one particular way, and extrapolation of the results to different food or different processing methods is not always possible.

7. The investigations to be carried out will cover the separation and identification of the most important volatile compounds and examination of non-volatile compounds (enzymes, vitamins, organic acids, carbohydrates, pectic substances, peroxides, carbonyls and sulphur compounds).

BIOLOGICAL SCREENING

8. The toxicity of any compounds of special interest isolated by chemical means will be investigated in special animal tests comprising other animals such as golden hamsters, guinea-pigs, monkeys and dogs. This would in particular be the case if substances can be isolated which show radiomimetic properties when tested on plant root meristems or tissue culture cells. The details of these experiments will have to be chosen according to the requirements in each individual case.

If fairly stable radiomimetic principles are found, they should be investigated at high concentrations in special tests aimed at determining the dose-response relationship for possible types of toxicity and histo-pathological changes. In this way the wholesomeness of the commercially irradiated product could be determined by an approach quite different from that of conventional wholesomeness tests with large groups of animals. If toxic or other effects are found at all at

high concentration, the hazards involved in consuming the product could be estimated by extrapolation to the actual concentration in the latter. Possibly, a threshold concentration may be found, below which no hazards will be met.

9. The animal tissue culture facilities of the Seibersdorf Institute may be used for estimating the « translation factor » to be used when the risks to man are determined from animal experiments. This can be done by comparing the sensitivity of human and animal cells to a radiomimetically active principle induced by food irradiation.

If radiomimetic compounds can be isolated these experiments should be continued with a view to solving the fundamental radiobiological problems of the action mechanisms of the active principles. This work will call for highly refined techniques on different biological systems, and will therefore require co-operation with specialised laboratories outside Seibersdorf.

10. It is a matter of general interest to establish whether irradiated food can produce gene mutation. Experiments to elucidate this point should be done with micro-organisms and may be carried out outside Seibersdorf.

To consider possible effects in mammals, model studies of, for example, recessive lethal mutations in Drosophila seem suitable. Studies of dominate lethal mutations in mice could be started simultaneously. Experiments should be made in order to establish whether radiomimetically active principles may be carried via the alimentary tract to important organs of the animal's body.

11. As a link in this additional fundamental research programme on radiomimetic or « indirect » radiation effects, the possibility of induced carcinogenicity should be elucidated using such techniques as the rapid skin test recently developed.

II. MICROBIOLOGY

12. The main problem of irradiation pasteurization of foods with a pH lower than 4.5 is the control of yeasts. Results of investigations on the radiation sensitivity of yeasts are already available. The natural sensitivity of yeasts varies widely. The dose giving the degree of inactivation required for pasteurization is approximately 0.2 Mrad for certain strains of baker's yeast, whereas some strains of wine yeast require 2 Mrad. The combination of irradiation with other treatments such as heat, the addition of vitamin K5 or an enrichment of the yeasts with

certain trace elements, makes it possible to reduce the lethal dose by a factor of 10. It is obvious that the total yeast count and environmental conditions will influence the dose required for the inactivation of yeasts and it will therefore be necessary to determine the sensitivity of yeasts in specific foods.

13. The investigation should be carried out in two steps, the first using fermentation of the substrate as indication of the survival of yeasts after irradiation. In relatively simple experiments the influence on yeast sensitivity can be studied of factors such as: origin of the food, chemical environment, yeast count, distribution of yeast species, temperature, atmosphere, total dose and dose rate.

A second step will certainly be the selection of a few typical yeast species for detailed studies of their radiation biology and the mechanism of radiation effects on the metabolism.

III. THE EFFECTS OF IRRADIATION ON THE QUALITY AND MANUFACTURING PROPERTIES OF FRUIT JUICES

14. Irradiation of fruit juices can cause changes in appearance and flavour and these two criteria will be used as a measure of the effect of various irradiation conditions.

The changes of colour are easily measured by means of a spectro-photometer. Substantial changes in flavour caused by irradiation can easily be detected by one person. For small changes in flavour, taste panel experiments will be required. To permit statistical appraisal of the results, these experiments will need to be repeated many times and are likely to extend over a considerable period. The results will serve as a basis for the inauguration of detailed bio-chemical work.

Using the above criteria, the following parameters will be investigated:

- Total dose and dose rate
- Irradiation conditions (temperature, atmosphere)
- Storage time and conditions (temperature, light, etc.)
- Pretreatment (heat and chemical)
- Variety, growing conditions, vintages, ripeness and pressing methods for the juice.

IV. STAFF REQUIREMENTS

15. The estimated staff requirements over the six years are likely to vary between a minimum of 7 and a maximum of 13 scientists in the first year and a minimum of 15 and a maxi-

mum of 24 scientists in the third year. Austria will provide 4 scientists to start with and up to approximately one-third of the scientists later on. Depending on the needs of the project, a suitable number of auxiliary staff, up to a maximum of 35, shall be provided entirely by Austria.

ANNEX II

INSTALLATIONS AND EQUIPMENT TO BE MADE AVAILABLE BY THE S. G. A. E.

1. *Main Building*
   - *(a)* Laboratories (848.9 m²)
   - *(b)* Livestock Department (253.6 m²)
   - *(c)* Offices (141.2 m²)
   - *(d)* Library, conference room and visitors' room (147.3 m²)
   - *(e)* Auxiliary areas including heating, ventilation, cloakrooms, etc. (1,076.1 m²).

2. *Equipment for Main Building*

3. *Irradiation Plant*

A concrete irradiation plant with an overall surface of about 15 m × 6 m will include two irradiation chambers each with a surface of about 10 m², one for 1,000 c and the other for 20,000 c.

4. *Cooling Plant*

A cooling plant of about 30 m² will supply the main building and the irradiation plant with cooling and air-conditioning.

DONE in Vienna, 16 September 1964, in the English and French languages.

For
Österreichische Studiengesellschaft
für Atomenergie GmbH

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