INTERNATIONAL CONFERENCE ON RADIATION RESEARCH
Radiation Laboratory for the Preservation of Food
Natick Laboratories, Natick, Massachusetts

WHOLESOMENESS OF IRRADIATED FOOD

Nicholas Raica, Jr., Ph.D.
and
Marion E. McDowell, Lt.Col., MC

U. S. Army Medical Research & Nutrition Laboratory
Fitzsimons General Hospital
Denver 30, Colorado

William J. Darby, M.D.
Vanderbilt University
School of Medicine
Nashville 5, Tennessee

Donald L. Eppie, Lt. Col. and Jacques L. Sherman, Jr., Lt. Col.
U. S. Army Medical Research and Development Command
Office of the Surgeon General
Washington 25, D. C.

International Conference on Radiation Research Commemorating the Operational Availability of the Radiation Laboratory for the Preservation of Food, Natick, Mass., January 14-16, 1963

The Principles of Laboratory Animal Care as Promulgated by the National Society for Medical Research were Observed.

DISCLAIMER

The availability of radioisotopes and radioactive by-products from nuclear reactors during the early post-war years prompted searches for methods of utilizing this energy. The U. S. Army Quartermaster Corps became interested in investigating the feasibility of preserving foods by sterilizing them with ionizing radiations and, accordingly, had initiated a comprehensive program toward this end (59, 85). Very little information was available in the early 1950's on the wholesomeness of irradiated foods. Consequently, as a part of the U. S. Army's overall program, the Medical Research Branch of The Surgeon General's Office was assigned in 1953 the task of determining the wholesomeness of "cold sterilized" foods. Since that time, a very comprehensive program has been supported by the QMC in civilian academic and research institutions as well as in military research installations. The progress of these wholesomeness studies, through the years, has been well documented (19, 59, 70, 79, 85).

While a final evaluation of the entire program at this time would be premature, a sufficient number of long-term generation feeding and carcinogenicity studies have been completed to permit a visualization of reported data in a perspective not possible a short time ago.

Time will not permit the presentation of the experimental details which have been adequately reported in the literature. However, brief mention will be made of the general outline followed in the various wholesomeness studies.

Procedural guides for the long-term toxicity studies, two years or four generations, and histopathology studies have been designed

through the cooperation of the Food and Drug Administration, Office of The Surgeon General and the Armed Forces Institute of Pathology (31, 56, 57, 58, 74). Briefly, the experimental animals (rats, dogs, monkeys and mice) are fed a diet containing the irradiated test food in a quantity greater than that food would normally be consumed in order to maximize any potential toxicity. In general, the food has received an irradiation dose of 2.79 and 5.58 Krads and is stored at room temperature for at least three months before incorporation into a normally adequate diet as 35% of total dietary solids. Certain foods such as potatoes, oranges and flour have received dosages of less than 500 Krads.

In support of the toxicity studies, numerous *in vivo* and *in vitro* investigations have been made on the nutritional adequacy and nutrient stability of irradiated foods. Unfortunately, time will not permit more than brief mention of these interesting and productive studies during this presentation on the wholesomeness of irradiated foods.

The parameters studied in the long-term toxicity studies are listed in table 1. For completeness, induced radioactivity should also be included; however, this problem can be studied apart from the feeding tests. Induced radioactivity investigations have been included in both the Quartermaster Corps and Surgeon General's programs. As a result of these and other studies, it is generally agreed that gamma rays from cobalt-60 or electrons of less than 10 Mev will not produce detectable induced radioactivity in foods (2, 17, 24, 32, 42, 43, 59).

Through short-term acute toxicity rat-feeding experiments (8-12 weeks) 40 foods have been cleared for long-term animal feeding and short-term human feeding and taste panel studies (3, 39,55, 61, 62, 80). Of these 40 foods, 21 have been chosen as representative to be used in the long-term toxicity studies. The foods and experimental animals are listed in table 2. Each food will be discussed individually, although in a few instances two foods were fed simultaneously in a 3 x 3 factorial design.

The first foods to be discussed are those which have received irradiation dosages of less than 300 Krads. They are listed in table 3 along with the principal investigator, irradiation dosage, test animal and results. A negative result indicates that no significant difference was observed between the animals eating irradiated or nonirradiated food as measured by the parameters listed in table 1.

Brownell et al. (7) reported a slight, but of questionable statistical significance, increase in the mortality of the first and second generation rats eating irradiated potatoes. This investigator felt that the rot which developed in the irradiated potatoes and genetic factors were of more significance than the irradiation in producing the slight increase in mortality. Kline et al. (28, 29) and McCay and Ramsey (36) reported no significant differences in similar studies with rats or dogs.

Flour was fed to rats by Buhl et al. (62) and to dogs by Reber et al. (65, 63). While no significant differences were reported by Buhl, Reber reported a slightly increased incidence of thyroiditis in dogs eating the irradiated flour. The thyroiditis found in beagles

is not related to irradiated foods. A study by Tucker (84) of thyroids from 167 dogs in the wholesomeness food program showed that while a high incidence (26.2%) of thyroiditis was found, its occurrence was equally distributed between male and female, irradiated and nonirradiated groups.

Oranges were fed by Phillips et al. (52, 53) to rats and by Blood and Darby (4) to monkeys. Phillips reported a decreased growth rate in the irradiated group and poor reproduction in both irradiated and nonirradiated groups eating a diet consisting of 22% (solids) whole oranges. This diet was not considered a good rat diet.

The next group of foods to be discussed are meats and fish irradiated to 2.79 and 5.58 Mrads as listed in table 4. Beef was fed to rats or dogs by Blood and Darby (5, 6), McCay and Ramsey (37) and Reber et al. (66, 69). The decreased fertility reported by McCay in dogs was believed to have been due to insufficient vitamin E supplementation. This experiment is being repeated by McCay as well as by Clarkson and Moreland (11). Recent reports from these investigators indicate that there appears to be no incidence of decreased fertility in the dogs eating 5.58 Mrad irradiated beef. There were no significant differences between irradiated and nonirradiated beef stew, pork, chicken, chicken stew, cod fish, tuna fish or shrimp when fed to rats or dogs by Deichmann (14), Blood and Darby (6), Tinsley et al. (81, 82), McCay and Ramsey (37), Richardson et al. (70), Salman and Newburne (1, 49, 50), Poynter (51), Phillips et al. (52, 54) and Engel et al. (20, 21).

Results of studies with vegetables, fruit and jam irradiated to 2.79 and 5.58 Mrads are listed in table 5. There were no significant differences between irradiated or nonirradiated carrots, green beans, cabbage, corn, sweet potatoes, peaches or pineapple jam when fed to dogs or rats by Engel et al. (20, 21), Richardson et al. (70), Hale et al. (22), Phillips et al. (54), McKay and Ramsey (38), Poynter (51), Tinsley et al. (82) and Salman and Norberne (1, 49, 50). Decreased growth and decreased carotene utilization were reported by Tinsley et al. (82) in rats fed irradiated carrots. This experiment is being repeated because the irradiated carrots were contaminated and in poor condition. The increased spleen weights observed by Larsen et al. (30) in dogs fed irradiated green beans or fruit compote may be of doubtful significance. Spleen weights in dogs are subject to rapid change through change in blood volume. The vitamin C deficiency noted by Blood and Darby (4) in monkeys fed irradiated peaches was correctable by vitamin C supplementation. Mead and Griffith (40) reported a possible decreased longevity in rats fed irradiated fruit compote and bacon. Statistical analysis of the data, as presented, showed that the differences in longevity were not significant. The glycosuria reported by Blood and Darby (6) in dogs fed irradiated jam was due to the high carbohydrate content of the diet. Fasting sugar levels were normal.

In table 6 are listed the bacon, bacon lipid and lard studies. No significant differences were reported by Hale et al. (23) in dogs fed irradiated bacon or by McKee et al. (18) in mice fed irradiated bacon lipid. The decreased longevity reported by Mead and Griffith (40) in

rats fed irradiated bacon was discussed and the difference was not significant. The experiment by Schreiber and Bassett (77) in which the decreased rate of digestion of irradiated lard was reported should be explained in more detail than the feeding experiments. In this study, lard or lard-meat mixtures were fed to dogs by intubation. After three hours, the animals were sacrificed. Stomach and intestinal contents were analyzed. The irradiated (5.50 Mrads) lard was retained in the stomach to a greater extent than the nonirradiated lard (65 vs. 49%), filled the small intestine to a lesser extent (9 vs. 13%) and was absorbed to a lesser extent (26 vs. 38%). Less absorption was also indicated from jejunal fistula collections over a 12-hour period. The peroxide number of the irradiated lard was 176 and continued to rise to over twice this value over a period of 14 months in cold storage. A peroxide number of 176 is much higher than that normally found in irradiated fats. Generally, irradiated fats have peroxide numbers of less than 100.

Oxidized soy oil with peroxide numbers (PN) of 100 and 400 was fed to rats by Mead and Griffith (40). They reported normal growth and food consumption in the PN 100 group and decreased growth and food consumption in the PN 400 group. However, reproduction and lactation in both (PN 100 and 400) groups were normal. Moore (47) reported that although the decreased digestibility of irradiated fat (corn oil) fed to rats (8 months) was statistically significant, "---it is doubtful whether the gain or loss of digested nutrients would be of biological significance." Ritchey and Richardson (73) reported equal growth and mortality when irradiated

or nonirradiated corn or soy oil was fed to chicks. When diets contained irradiated or nonirradiated pork fat or irradiated beef fat, poor growth and high mortality resulted. Normal growth and mortality resulted when the chicks were fed animal fats containing an antioxidant (BHT) or when they were given oral supplements of vitamins A and D. Monty (46) using methyl carbonyls reported that while carbonyls would inhibit *in vitro* lipase activity and depress the rate of fat absorption *in vivo*, they had no effect on net absorption during a 20-day feeding trial. The aldehyde was absorbed and metabolized to a significant degree.

The above studies indicate that aldehyde formation in irradiated fats may not be detrimental; however, peroxide formation may be a problem particularly if the peroxide number rises to more than 100. This aspect deserves further work, although it is not pertinent to the wholesomeness study.

Dried whole egg (table 6) irradiated to 0.93 and 2.78 Mrad was fed to dogs by Proctor et al. (60). Decreased fertility was reported in both the irradiated and nonirradiated groups. Further investigation by Proctor determined that the decreased fertility was due to a biotin deficiency caused by the avidin content of the raw egg preparation. No significant differences were reported by Deichmann (15) in rats or dogs fed irradiated or nonirradiated evaporated milk (table 6).

In addition to the one or two irradiated food component diets reported above, the U. S. Army National Research and Nutrition Laboratory conducted a long-term toxicity study in rats fed a composite diet of 9 foods, each irradiated to 5.59 Mrads and stored frozen until fed (64). The foods used in this study are shown in table 7. Nonirradiated

vitamins and minerals were added to the irradiated and nonirradiated diet. No significant differences were found which could be attributed to consuming a diet supplying 100% of the calories as irradiated foods.

As a further test of wholesomeness, studies in tumor-susceptible strains of mice were initiated. These special carcinogenicity studies are listed in table 8.

Kline and Tuply (27) reported a study designed to test the possible formation of carcinogens upon irradiation of crude sterol-containing preparations. The preparations were a mixture of fresh pork brain and egg, a mixture of sterol concentrates, a mixture of vegetable oils, a mixture of ground meat-fish-cheese-milk powder, and lard. Extracts were fed, painted and injected into rats and mice. No evidence of carcinogen formation was found in the irradiated preparations.

Studies by Calandra and May (9) and Deichmann (16) with composite diets are almost completed. Data to date from both studies indicate no significant differences between the irradiated and nonirradiated groups.

Monson (45) in a carcinogenicity study in mice fed an irradiated composite diet of pork, chicken, evaporated milk, potatoes and carrots reported a peculiar cardiac lesion consisting of a dilatation of the left auricular appendage, which sometimes ruptured. After 600 days on diet, nearly 50% of one strain on the irradiated diet had such lesions as compared to a much lower incidence among those on the nonirradiated diet. In subsequent experiments, Monson was able to reproduce this lesion in mice with either a diet of irradiated or nonirradiated milk. This lesion is still being studied by Monson as well as by the Pathology

vision of the U. S. Army Medical Research and Nutrition Laboratory under the direction of Lt. Col. Thompson. The study by Thompson is outlined in table 9. It includes genetic, pathogenesis and etiology studies in addition to a repeat of Hansen's experiment. The experimental feeding has been completed and a final report is expected some time this spring.

No gross heart lesions as described by Hansen occurred in this study. To date, with histopathology completed on the tissues of 3400 (70%) of the mice, there has been no unusual overall incidence of lesions. However, it is to be emphasized that these evaluations are made and recorded without knowledge of the group to which the animal belongs; thus, final conclusions must await decoding of the experiment after all tissues are read.

In addition to the histopathological examination of tissues from animals in the long-term feeding experiments conducted locally by pathologists associated with each investigating group, the necropsy reports and a complete set of tissue sections obtained and blocks of paraffin-imbedded tissues are forwarded to the Armed Forces Institute of Pathology in Washington 25, D. C. for review and compilation of a separate overall report (75). Most of the individual studies have been completed. No evidence has been noted from these reviews to suggest that irradiation of the food produced any histopathologic changes. A final summary report for each species will be prepared some time during 1963.

Short-term human feeding studies with irradiated foods were conducted at the U. S. Army Medical Research and Nutrition Laboratory during 1957 and 1958 (39, 53). Seven separate feeding tests were carried

out, each with 9 or 10 volunteer test subjects. A total of 33 men (19-24 years of age) participated in the 15-day tests. Fifty-four foods irradiated from 10 Krads to 4 Mrads and stored frozen or at room temperature for three months were fed up to 100% of total daily calorie intake. There was no evidence of toxic effects from eating irradiated foods in any of the men either by clinical or laboratory tests. Digestibilities of the macronutrients and metabolizable energy were similar in the irradiated and nonirradiated diets.

In addition to the short-term human feeding tests, a study of the acceptability of irradiated (sterilized) pork and bacon was made at Fort Lee, Virginia in 1959 (8). The test foods were served as a part of a normal meal under garrison feeding conditions to 120 volunteers during each test. Sixty were served irradiated food and 60 were served "fresh" nonirradiated food. The irradiated pork loin had been stored at room temperature for 10 months and the irradiated bacon was stored at room temperature for 1 year prior to feeding. Conclusions of the tests were that the radiation preserved foods were as acceptable as their respective standard nonirradiated foods.

Long-term human feeding studies probably will not be initiated before completion of the investigations currently in progress and all data are accumulated and evaluated for the wholesomeness program in its entirety.

A review of the possible unfavorable findings in the long-term feeding studies is presented in table 10 and will be commented upon again briefly.

1. The thyroiditis in dogs is a nonspecific lesion which has been reported to occur with equal frequency in irradiated and nonirradiated dietary groups.

2. Decreased growth in rats fed the irradiated whole orange diet is probably due more to the "abnormal" diet than to irradiation. Both irradiated and nonirradiated groups had poor breeding performance. Pealed oranges did not show these differences.

3. The decreased longevity in rats fed irradiated bosc-fruit compote diet was not substantiated when the data were analyzed statistically.

4. The decreased rate of fat digestion and absorption in dogs fed irradiated lard has not been sufficiently studied for adequate interpretation. However, it should be kept in mind that these were acute studies (3-12 hours) and that the lard had an abnormally high peroxide number (170-340).

5. The increased spleen weights in dogs fed irradiated fruit compote or green beans may not be of significance since spleen weights are subject to rapid change.

6. The decreased growth rate of rats fed irradiated carrots may have been due to microbial contamination. This study is being repeated and should be completed in the near future.

7. The decreased fertility in dogs fed irradiated beef was probably due to insufficient vitamin E supplementation. Preliminary reports from repeat studies indicate normal reproductive performance.

8. The degenerative heart lesion in mice has been reproduced with nonirradiated diets. Studies designed to study this lesion are nearing

completion and until all the data are analyzed, no firm statement can be made at this time of its significance.

Before concluding this presentation on the wholesomeness of irradiated foods, mention should be made of some of the very interesting nutritional findings which have been a direct result of the U. S. Army Surgeon General's wholesomeness program.

The most fruitful development has been that in vitamin K nutrition. Metta, Hamaoch and Johnson in 1959 (43) were the first to demonstrate that the rat, contrary to current thoughts at that time, does require a dietary source of vitamin K. The diet used by these investigators was an irradiated beef diet which was suboptimal in vitamin K. Spontaneous fatal hemorrhages resulted primarily in the irradiated beef dietary group. Subsequent investigations by Johnson's group and others established the following factors bearing on vitamin K nutrition that may have an influence on the requirement for vitamin K.

1.	Protein sources	(19, 26, 34, 63)
2.	Amino acids	(19, 33)
3.	Coprophagy	(26)
4.	Strain, age, sex	(41, 44)
5.	Species	(25, 67, 76)
6.	Vitamins A and E	(33, 41)
7.	Bile acids	(63)
8.	Lipids	(19)
9.	Aldehydes	(26)

More recently, Schendel and Johnson (76) have established a dietary requirement for the baby pig. Of more interest than the establishment of a dietary requirement for vitamin K in the pig are the symptoms shown in deficiency. Only slightly elevated prothrombin times were observed during the course of the experiment. Shortly before the sudden death which occurs, there develops anorexia, anemia and hypersensitivity of the skin. No evidences of internal hemorrhaging were observed. Vitamin K administration reversed the observed symptoms and restored normalcy. Experiments by this group with monkeys suggest that while monkeys do have a dietary requirement for vitamin K, they are apparently able to utilize directly intestinally synthesized vitamin K (85).

During the evolution of the vitamin K-irradiated foods problem, the terms hemorrhagic or hemorrhagenic diet were applied to Johnson's irradiated beef diet which was not supplemented with vitamin K. Consequently, any vitamin K-free or deficient diet, whether it be a non-irradiated soy protein or non-irradiated pork diet, which causes hemorrhaging due to a lack of vitamin K has also been termed a hemorrhagenic diet. This is very misleading since hemorrhagenic connotes an unknown or known toxic factor which has not been established. These diets should be referred to what they are - vitamin K-free or deficient diets.

In conclusion, it is evident from the tremendous volume of data accumulated from the wholesomeness studies, with the exception of one or two doubtful areas, that foods irradiated to 5.90 krads are wholesome. Their nutritional adequacy is comparable to conventional heat-processed foods. This adequacy has been demonstrated in reports to The Surgeon General's Office and other publications (10, 12, 13, 47, 53, 71, 72, 83, 85, 86).

The evaluation of the [illegible] program should wait until all data, feeding and histopathologic, have been reviewed. In the meantime, certain foods which have consistently shown acceptable standards of wholesomeness, palatability and promising adaptability are being considered for acceptance by the U. S. Army for further concentrated studies in the near future.

REFERENCES

1. Alexander, H. D. and W. D. Salmon. Long-term rat and dog feeding tests on irradiated sweet potatoes and cod fish. Progress report 15 (Final on rat and dog feeding tests). Contract No. DA-49-007-MD-543 with OTSG. September 1959.

2. Barnes, J. M. et al. Radiation-processed foods as a component of the Armed Forces feeding systems Operations Research Office. The Johns Hopkins University. ORO-SP-174, August 1961.

3. Benham, C. H., F. Minsenburger and R. Ehrlich. Subacute toxicity of irradiated foods. Final report. Contract No. DA-49-007-MD609 with OTSG. October 1959.

4. Blood, F. R., W. J. Darby and others. Long-term monkey feeding experiment on irradiated peaches, whole oranges and peeled oranges. Final report, Section I. Contract No. DA-49-007-MD-779 with OTSG. 1961.

5. Blood, F. R., W. J. Darby and others. Long-term rat feeding experiment with irradiated beef. Final report. Section I. Contract No. DA-49-007-MD-779 with OTSG. 1961.

6. Blood, F. R., W. J. Darby and others. Long-term dog feeding experiment with irradiated chicken, beef and jam. Final report. Section I. Contract No. DA-49-007-MD-779, with OTSG. 1961.

7. Brownell, L. E., C. H. Burns and G. D. Abrams. Wholesomeness of gamma-irradiated diets. Final report. Growth, reproduction, mortality and pathologic changes in rats fed gamma-irradiated potatoes. Contract No. DA-49-007-MD-581 with OTSG. February 1959.

8. Burt, T. B. et al. Troop acceptability tests of radiation preserved pork and bacon. Technical report T-81, 57029-F. QM Field Evaluation Agency, U.S. Army QM Research and Engineering Command. Fort Lee, Va., September 1958.

9. Calandra, J. C. and J. H. Kay. The carcinogenic properties of irradiated foods. Progress reports 4 and 7. Contract No. DA-49-007-MD-895 with OTSG. August 1959 and February 1961.

10. Calloway, D. H. and M. H. Thomas. Nutrient content and processing characteristics of irradiated foods used in long-term animal-feeding studies. QM Food and Container Institute for the Armed Forces. Report No. 17-61, August 1961.

11. Clarkson, T. B. and A. F. Moreland. The effect of control-ground beef and irradiated 5.58 megarad-ground beef consumption on reproductive performance in the beagle. Progress report 4. Contract No. DA-49-193-MD-209A with OTSG. March 1962.

12. Day, E. J., H. D. Alexander, H. E. Sauberlich and W. D. Salmon. Effects of gamma-irradiation on certain water soluble vitamins in raw ground beef. J. Nutrition, 62: 27- (1957).

13. Day, E. J., H. E. Sauberlich, H. D. Alexander and W. D. Salmon. The bioassay of thiamine in beef exposed to gamma-radiation. J. Nutrition: 62: 107- (1957).

14. Deichmann, W. B. Long-term dog and rat feeding experiment employing irradiated beef stew (C ration). Final report. Contract No. DA-49-007-MD-785 with OTSG. August 1961.

15. Deichmann, W. B. Long-term dog and rat feeding experiment employing irradiated evaporated milk.. Final report. Contract No. DA-49-007-MD-785 with OTSG. August 1961.

16. Deichmann, W. B. Mouse carcinogenicity study. Progress report 8. Contract No. DA-49-007-MD-789 with OTSG. March 1962.

17. Desrosier, N. W. and H. M. Rosenstock. Radiation Technology in Food, Agriculture and Biology. The Avi Publishing Co., Inc., Westport, Connecticut, 1960.

18. Dixon, M. S., D. L. Meyer, L. J. Zeldis and R. W. McKee. Influence of irradiated bacon lipids on body growth, incidence of cancer and other pathologic changes in mice. J. Food Sci., 26, 611-617 (1961).

19. Doisy, E. A., Jr. Nutritional hypoprothrombinemia and metabolism of vitamin K. Federation Proceedings, 20: 989-994 (1961).

20. Engel, R. W. and D. F. Watson. Long-term dog feeding of irradiated and control shrimp and carrots (phase 1) and nutritive value of irradiated and control proteins (phase 2). Progress report 5 (growth data). Contract No. DA-49-007-MD-784. September 1959.

21. Engel, R. W., D. F. Watson and W. F. Mastansa. Long-term dog feeding of irradiated and control shrimp and carrots (phase 1) and nutritive value of irradiated and control proteins (phase 2). Progress report 6 (Histopathology of shrimp fed dogs). March 1960.

22. Hale, M. W., W. F. Schroeder and D. Sikes. Growth, reproduction, mortality and pathologic changes in dogs fed gamma-irradiated cabbage for two years. Final report. Contract No. DA-49-007-MD-780 with OTSG. March 1960.

23. Hale, M. W., W. F. Shroeder and D. Sikes. Growth, reproduction, Mortality and pathologic changes in dogs fed gamma-irradiated bacon for two years. Final report. Contract No. DA-49-007-MD-780 with OTSG. January 1960.

24. Hannan, R. S. Research on the science and technology of food preservation by ionizing radiations. Chemical Publishing Co., Inc. New York, N. Y., 1956.

25. Hill, R. B., S. Gaetani, and B. C. Johnson. On the nutritive value of the major nutritients of irradiated foods. Progress report 22. Contract No. with OTSO, DA-49-007-MD-544. September 1962.

26. Johnson, B. C., M. S. Mameesh, V. C. Metta and P. B. Rama Rao. Vitamin K Nutrition and irradiation sterilization. Fed. Proc., 19: 1038-1044 (1960).

27. Kline, B. E. and L. J. Topley. The possible carcinogenicity of irradiated foods. Final report. Contract No. DA-49-007-MD-583 with OTSO. December 1959.

28. Kline, B. E., H. von Elbe and J. J. Birdsall. Long-term feeding of irradiated potatoes. Final report. Part I. Growth and reproduction. Contract No. DA-49-007-MD-712 with OTSO. November 1960.

29. _____, _____ _____. Ibid. Part II. Pathology, January 1961.

30. Larson, P. S., L. F. Balter, E. M. Crawford, H. B. Haag, J. K. Finnegan and R. B. Smith, Jr. Effects of adding gamma-irradiated green beans or fruit compote to the diet of dogs. Toxicol. Appl. Pharmacol. 3: 57-62 (1961).

31. Lehman, A. J. and E. P. Laug. Evaluating the safety of radiation-sterilized foods. Nucleonics 12: 52-54 (1954).

32. Leveque, Pierre. Threshold for induced radioactivity in irradiated foods. Food Irradiation 1, 9-10 (1960).

33. Malhotra, O. P. and E. F. Reber. Effects of methionine on blood coagulation in rats fed a vitamin K deficient ration. Fed. Proc. 20: 55 (1961).

34. Mameesh, M. S. and B. C. Johnson. Production of dietary vitamin K deficiency in the rat. Proc. Soc. Exper. Biol. & Med. 101: 467-468 (1959).

35. Matshiner, J. T. and E. A. Doisy, Jr. Role of vitamin A in induction of vitamin K deficiency in the rat. Proc. Soc. Exper. Biol. & Med., 109: 139 (1962).

36. McCay, C. M. and G. L. Ramsey. Effect of ionized radiation on the nutritive value of food. (Potatoes) Final report. Contract No. DA-49-007-MD-680 with OTSO. March 1960.

37. McCay, C. M. and G. L. Rumsey. Effect of irradiated meat upon growth and reproduction of dogs. Fed. Proc. 19: 1027-1030 (1960).

38. McCay, C. M. and G. L. Rumsey. Effect of ionized radiation on the nutritive value of food (corn) as determined by growth, reproduction and lactation studies with dogs. Final report. Part I. Contract No. DA-49-007-MD-600 with OTSO. March 1960.

39. McCary, V. E. and M. E. Shipman. Acceptability of irradiated foods. II. J. Am. Dietet. Assoc., 32: 1059-1063 (1956).

40. Mead, J. F. and W. H. Griffith. Effect of ionizing radiation on the nutritive and safety characteristics of food. Final report. Contract No. DA-49-007-MD-579, with OTSO. September 1959.

41. Mellette, S. J. and L. A. Leone. Influence of age, sex, strain of rat and fat soluble vitamins on hemorrhagic syndromes in rats fed irradiated beef. Fed. Proc., 19: 1045-1049 (1960).

42. Meneely, G. R., R. L. Weiland and R. G. Hagstrom. Radioisotopes in radiation processed foods. Progress report 3. Contract No. DA-49-193-MD-2101 with OTSO. September 1961.

43. Metta, V. C., M. S. Maneesh and B. C. Johnson. Vitamin deficiency in rats induced by feeding irradiated beef. J. Nutrition 69: 18 (1959).

44. Metta, V. C. and B. C. Johnson. Effect of feeding vitamin K-deficient diets to female rats. J. Nutrition, 72: 455-458 (1960).

45. Monsen, H. Heart lesions in mice induced by feeding irradiated foods. Fed. Proc., 19: 1031-1034 (1960).

46. Monty, K. J. Carbonyl compounds as inhibitors of lipid metabolism and their significance in irradiated foods. Fed. Proc., 19: 1034-1037 (1960).

47. Moore, R. O. The influence of irradiated foods on the enzyme systems concerned with digestion. Final report. Contract No. DA-49-007-MD-787 with OTSO. April 1961.

48. National Academy of Sciences - National Research Council. Radionuclides in foods. Publ. 988. Washington, D. C. 1962.

49. Newberne, P. M. Final report on histopathology of rats fed codfish and sweet potatoes. Contract No. DA-49-007-MD-543 with OTSO. June 1960.

50. Newberne, P. M. Final report on histopathology of long-term dog feeding tests on irradiated codfish and sweet potatoes. Contract No. DA-49-007-MD-543 with OTSO. October 1960.

51. Paynter, O. E. Long-term feeding and reproduction studies on irradiated corn and tuna fish. Final report. Contract No. DA-49-007-MD-788 with OTSG. September 1959.

52. Phillips, A. W., H. R. Newcomb and D. Shanklin. Long-term rat feeding studies. Final report (shrimp and oranges). Contract No. DA-49-007-MD-791 with OTSG. June 1961.

53. _____, _____ _____. Ibid. Final report (irradiated oranges). June 1961.

54. Phillips, A. W., H. R. Newcomb and D. Shanklin. Long-term rat feeding studies: Irradiated chicken stew and cabbage. Final report. Contract No. DA-49-007-MD-783 with OTSG. August 1961.

55. Plough, I. C., E. L. Bierman, L. M. Levy and N. F. Witt. Human feeding studies with irradiated foods. Fed. Proc., 19: 1052-1054 (1960).

56. Procedural guide for conducting long-term feeding experiments on irradiated foods. Based on procedures recommended by Dr. F. E. Rice (consultant to OTSG), Drs. E. A. Brownlee and J. W. Pratt (University of Chicago Statistical Group), and Food and Drug Administration.

57. Procedural guide for conducting dog feeding experiments for coordinated studies on radiation processed foods. Compiled from information obtained from OTSG irradiated food contractors, consultants to OTSG, and staff of the Food and Drug Administration.

58. Procedural guide for coordinated histopathological studies association with long-term feeding projects under the irradiation preservation and food program. OTSG, 15 August 1956.

59. Proceedings, Seventh Contractors' Meeting, QM Corps, Radiation Preservation of Foods Project, Chicago, Ill., June 1961. QM Food and Container Institute for the Armed Forces, 1819 W. Pershing Road, Chicago 9, Ill.

60. Proctor, B. E., S. A. Goldblith and S. A. Miller. Research to determine whether any toxic effects or protein quality changes are induced in dried whole egg by ionizing radiation. Final report. Contract No. DA-49-007-MD-755 with OTSG. June 1960.

61. Read, M. S., H. F. Kraybill and N. F. Witt. Short-term rat-feeding studies with gamma-irradiated food products. I. Frozen stored foods. J. Nutrition: 65: 39-52 (1958).

62. Read, M. S., H. M. Trabosh, W. S. Worth, H. F. Kraybill and N. F. Witt. Short-term rat feeding studies with gamma-irradiated food products. II. Beef and pork stored at elevated temperature. Toxicol. Appl. Pharmacol. 1: 417-425 (1959).

63. Read, M. S. Current aspects of the wholesomeness of irradiated food. J. Agric. Food Chem., 8: 342-349 (1960).

64. Read, M. S., H. F. Kraybill, W. S. Porth, S. W. Thompson, II, G. J. Isaac and N. F. Witt. Successive generation rat feeding studies with a composite diet of gamma-irradiated foods. Toxicol. Appl. Pharmacol. 3: 153-173 (1961).

65. Reber, E. F., O. P. Malhotra, J. P. Kreier, H. W. Norton and P. D. Beamer. The effects of feeding irradiated flour to dogs. I. Growth. Toxicol. Appl. Pharmacol. 1: 55-60 (1959).

66. Reber, E. F., O. P. Malhotra, J. P. Kreier, H. W. Norton and P. D. Beamer. The effects of feeding irradiated beef to dogs. I. Growth. Am. J. Vet. Res. 21: 367-370 (1960).

67. Reber, E. F., O. P. Malhotra, H. W. Norton and J. Simon. To determine the effect of irradiation upon the wholesomeness of food. Progress report 11. Contract No. DA-49-007-MD-728. March 1961.

68. Reber, E. F., O. P. Malhotra, J. Simon, J. P. Kreier, P. D. Beamer and H. W. Norton. Ibid. II. Reproduction and pathology. Toxicol. Appl. Pharmacol., 3: 568-573 (1961).

69. Reber, E. F., O. P. Malhotra, P. D. Beamer, H. W. Norton and J. P. Kreier. The effects of feeding irradiated beef to dogs. II. Reproduction and pathology. Am. J. Vet. Res. 23: 74-76 (1962).

70. Richardson, L. R., S. J. Ritchey and R. H. Rigdon. A long-term feeding study of irradiated foods using rats as experimental animals. Fed. Proc. 19: 1023-1027 (1960).

71. Richardson, L. R., S. Wilkes and S. J. Ritchey. Comparative vitamin B_6 activity of frozen irradiated and heat-processed foods. J. Nutrition, 73: 363-368 (1961).

72. Richardson, L. R., S. Wilkes and S. J. Ritchey. Comparative vitamin K activity of frozen, irradiated and heat-processed foods. J. Nutrition: 73: 369-373 (1961).

73. Ritchey, S. J. and L. R. Richardson. The effect of irradiated vegetable oils and animal fatty tissue and storage of the diet on growth and mortality in chicks. Poultry Science, 39: 404-408 (1960).

74. Ross, M. A. and E. C. Hood. Numerical codes for evaluation of pathologic findings in animals fed foods sterilized by irradiation. AFIP, August 1959 (Limited distribution).

75. Moss, W. A. Armed Forces Institute of Pathology, Washington 25, D. C. U. S. Army P & D Project No. 6X60-11-001.

76. Schendel, H. E. and B. C. Johnson. Vitamin K deficiency in the baby pig. J. Nutrition, 76: 124-130 (1962).

77. Schreiber, M. and E. S. Nasset. Digestion of irradiated fat in vivo. J. Appl. Physiol., 14: 639-642 (1959).

78. Symposium on Nutritional and Toxicological Studies on Irradiated Foods. Fed. Proc. 15: 905-937 (1956).

79. Symposium on New Aspects of Nutrition Uncovered in Studies with Irradiated Foods. Fed. Proc. 19: 1023-1059 (1960).

80. Teply, L. J. and B. E. Kline. Wholesomeness and possible carcinogenicity of radiated foods. Fed. Proc., 15: 927-929 (1956).

81. Tinsley, I. J., Bubl, E. C. and J. F. Bone. Tissue modification and irradiated food ingestion. Fed. Proc., 19: 1049-1052 (1960).

82. Tinsley, I. J., E. C. Bubl., J. S. Butts and J. F. Bone. The growth, breeding, longevity and histopathology of rats fed irradiated or control diets. Final report. Contract No. DA-49-007-MD-580 with OTSG. September 1961.

83. Thomas, M. H. and D. H. Calloway. Nutritional evaluation of dehydrated foods and comparison with foods processed by thermal and radiation methods. QM Food and Container Institute for the Armed Forces. Report No. 2-61, March 1961.

84. Tucker, W. E. Jr. Thyroiditis in a group of laboratory dogs. Am. J. Clin. Path. 38: 70-74 (1962).

85. U. S. Army Quarter Master Corps. Radiation Preservation of Food. U.S. Army Research & Development Series No. 1. Washington . U. S. Government Printing Office, August 1957.

86. Ziporin, Z. Z., H. F. Kraybill and H. J. Thach. Vitamin content of foods exposed to ionizing radiations. J. Nutrition 63: 201-210, (1957).

TABLE 1.--General Plan for Long-Term Toxicological Studies

Test Animals:	Rats, Dogs, Monkeys, Mice
Feeding Period:	Two years or four generations
Parameters:	Growth
	Reproduction
	Hematology
	Life span
	Histopathology
	Carcinogenicity

TABLE 2.--Listing of 21 Representative Foods Currently under Investigation in the U. S. Army's Irradiated Food Preservation Program

Food tested	Test animal		
	Rat	Monkey	Dog
1. Beef, ground	x		x
2. Pork loin	x		x
3. Bacon	x		x
4. Shrimp	x		x
5. Cod fish	x		x
6. Chicken	x		x
7. Tuna	x		x
8. Beef stew	x		x
9. Chicken stew	x		x
10. Carrots	x		x
11. Cole slaw	x		x
12. Corn	x		x
13. Beans, green	x		x
14. Potatoes	x		x
15. Potatoes, sweet	x		x
16. Flour	x		x
17. Fruit compote	x		x
18. Evaporated milk	x		x
19. Peaches	x	x	
20. Oranges	x	x	
21. Jam	x		x

TABLE 3.--Radiation Processed Feed, Long Term Feeding Studies

	Investigator	Radiation Kilorads	Animal	Results
Potatoes	Brownell, Michigan	13 - 20 27 - 40	Rats	Slightly increased mortality 1st and 2nd generation
	Kline, Wisc. Alum. Res.	7.5 - 8.5 15 - 17	Rats	Negative
	Kelby, Carroll	7 15	Dogs	Negative
Flour	Dahl, Tinsley, Oregon State	37 74	Rats	Negative
	Lehar, U. of Illinois	37 74	Dogs	Negative (? hypothalamic)
Oranges	Phillips, Syracuse	140 280	Rats	(Females) Negative (Males) Decreased growth
	Blood, Vanderbilt	140 280	Monkeys	Negative

TABLE 4.--Radiation Processed Food, Long Term Feeding Studies, 2.79 & 5.58 Mrads

Investigator	Animal	Results
Beef:		
Blood, Vanderbilt	Rats	Negative
McCay, Cornell	Dogs	Decreased fertility (? Vit. E)
McCay, Cornell	Dogs*	Experiment incomplete
Reber, U. of Illinois	Dogs	Negative
Clarkson, Bozeman-Gray	Dogs*	Experiment incomplete
Beef stew:		
Deichmann, U. of Miami	Rats	Negative
Pork:		
Tinsley, Oregon State	Rats	Negative
McCay, Cornell	Dogs	Negative
Chicken:		
Blood, Vanderbilt	Dogs	Negative
Richardson, Texas Ag. Expt. Sta.	Rats	Negative
Chicken stew:		
Phillips, Syracuse	Rats	Negative
McCay, Cornell	Dogs	Negative
Codfish:		
Salmon & Heyberne, Auburn	Rats	Negative
" "	Dogs	Negative
Tuna fish:		
McCay, Cornell	Dogs	Negative
Poynter, Embleton Labs.	Rats	Negative
Shrimp:		
Phillips, Syracuse	Rats	Negative
Watson, Va. Polytech. Inst.	Dogs	Negative (? Thyroiditis)

*5.58 Mrads.

TABLE 5.--Radiation Processed Food, Long Term Feeding Studies, 2.79 & 5.58 Mrads

Investigator	Animal	Results
Carrots:		
Engel, Va. Polytech. Inst.	Dogs	Negative
Tinsley, Oregon State	Rats	Decreased growth
Green beans:		
Richardson, Texas Ag. Expt. Sta.	Rats	Negative
Larson, Med. College of Va.	Dogs	Increased spleen weights
Cabbage:		
Sale, Ga. Coastal Plain Expt. Sta.	Dogs	Negative
Phillips, Syracuse	Rats	Negative
Corn:		
McCay, Cornell	Dogs	Negative
Payuter, Hazleton Labs.	Rats	Negative
Sweet potatoes:		
Salmon & Rayborne, Auburn	Rats	Negative
" "	Dogs	Negative
Peaches:		
Hood, Vanderbilt	Monkeys	Negative but Vit. C needed
Tinsley, Oregon State	Rats	Negative
Fruit compote:		
Mead, U.C.L.A.	Rats	Negative (? Decreased longevity)
Larson, Med. College of Va.	Dogs	Increased spleen weights
Pineapple jam:		
Hood, Vanderbilt	Dogs	Glycosuria
Tinsley, Oregon State	Rats	Negative

TABLE 6.--Radiation Processed Food, Long Term Feeding Studies, 2.79 & 5.58 Mrads

Investigator	Animal	Results
Bacon:		
Hale, Georgia Coastal Plain Expt. Sta.	Dogs	Negative
Mead, U.C.L.A.	Rats	Negative (? Decreased longevity)
Bacon lipid:		
McKee, U.C.L.A.	Mice	Negative in carcinogenicity study
Lard:		
Kasset, Rochester	Dogs	Decreased rate of digestion
Dried whole egg:*		
Proctor, Mass. Inst. Tech.	Dogs	Poor fertility
Evaporated milk:		
Brickman, U. of Miami	Rats	Negative
	Dogs	Negative

*1 and 3 Mrads.

TABLE 7.—Formulation of the Irradiated Composite Diet, 5.58 Mcals, Fed to Rats

	Dry solids, %
Bacon	8.8
Beef, ground	8.4
Haddock	19.2
Ham, fresh	4.8
Beans, green snap	8.4
Beets	15.4
Peaches	20.5
Cereal, military	3.0
Milk, powdered whole	5.6
Minerals, Hawk-Oser Number 3	2.9
Roughage	2.0
Vitamin-methionine-choline premix	1.0

Average per cent moisture = 77.7
Kilocalories per gram, wet = 1.14

USAFSAM

TABLE 8.--Radiation Processed Food, Carcinogenicity Studies

Investigator	Food	Animal	Results
As mentioned in previous tables		Rats	Negative
Kline & Teply, Wisc. Alum. Res. Found.	Pork brain & egg Sterol concentr. Vegetable oils Ground meat Fish, cheese Milk, lard	Mice Rats	Negative
McKee & Zeldis, U.C.L.A.	Bacon lipids	Mice	Negative
Calandra & Kay, Ind. Bio-Test Labs.	Cod fish Green beans Chicken stew Beef stew Peaches, flour	Mice	Unfinished, but negative to date
Deichmann, U. of Miami	Beef, corn Tuna fish Sweet potatoes Fruit compote	Mice	Unfinished, but negative to date

TABLE 9.--Outline of [illegible] Degenerative Heart Lesion Study

Composite diet: Pork, Chicken, Evaporated Milk, Potatoes, Carrots

Phase I - Breeding of all experimental animals - Parent generation maintained for life span.

Individual genetic records kept.

Phase II - Repeat of Hansen's experiment - with 2 control groups.

Animals sacrificed at 200, 300, 400, 500, 600 days.

Phase III - Pathogenesis study - Another group, fed only irradiated composite diet.

Animals sacrificed at 25-day intervals up to 400 days on diet.

Phase IV - Etiology study - a) 20 diets: One out of 5 items irradiated in each, divided as to whether diet is cooked, uncooked, with vitamin and without vitamin supplement, b) Milk-storage temperature varied, c) Milk diet alone.

Animals sacrificed at 100, 200, 400, 600 days.

TABLE 10.--Radiation Processed Food, Review of Remaining Unfavorable Findings

	Irradiated Food	Animal	Observation	Investigator
1.	Flour	Dogs	Thyroiditis (small number)	Reber
2.	Whole oranges	Rats	Decreased growth, 3rd generation	Phillips
3.	Bacon and fruit compote	Rats	? Decreased longevity	Mead
4.	Fats (lard)	Dogs	Decreased rate of digestion	Bassett
5.	Fruit compote (& green beans)	Dogs	Increased spleen weights	Larson
6.	Carrots	Rats	Decreased growth rate	Tinsley
7.	Beef	Dogs	Decreased fertility	McCay
8.	Composite diet	Mice	Heart degen. lesion	Monsen