

May 14, 2001

Mr. Thomas Billy  
Chairman, Codex Alimentarius Commission  
United States Department of Agriculture  
331 E Jamie Whitten Bldg  
Washington, DC 20250-3700

**RE: CX/FAC/01/11, Proposed Draft Revision to the Codex General Standard For Irradiated Foods; and USDA Docket No. 01-001N (Codex Alimentarius Commission; Thirty-Third Session of the Codex Committee on Food Additives and Contaminants)**

Dear Mr. Billy,

The Center for Food Safety (CFS) and Public Citizen are pleased to submit this public comment on the above-referenced Proposed Draft Revision to the Codex General Standard for Irradiated Foods, which the Committee on Food Additives and Contaminants (CCFAC) forwarded to the Commission pursuant to its meeting of 12-16 March, 2001, in The Hague.<sup>1</sup> CFS is a national, non profit, membership organization established in 1997 to use science and the law to address increasing concerns over the impacts of the global food production system on human health, animal welfare, and the environment. Public Citizen is a national, non-profit, membership organization established in 1971 that advocates for consumer protection and for government and corporate accountability.

## **EXECUTIVE SUMMARY OF COMMENT**

CFS and Public Citizen **oppose** the proposed revision of the Codex standards. The bracketed revision would remove the existing 10 kiloGray (kGy) irradiation maximum average absorbed dose limit. Numerous unresolved concerns remain related to the safety and wholesomeness of irradiated food. Recently, 26 medical experts endorsed a detailed warning in a prominent health journal on the dangers of food irradiation.<sup>2</sup> At least **ten positive *in vivo* published studies** that found mutagenic effects in mammals - including one in humans - were misclassified or ignored in the report upon which the CCFAC explicitly relied, the 1999 FAO/IAEA/WHO Technical Report #890. *High-Dose Irradiation: Wholesomeness of Foods Irradiated Above 10 kGy*, WHO,

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<sup>1</sup> This was listed as Agenda Item 9a (CX/FAC 01/11) at the CCFAC meeting.

<sup>2</sup> Epstein, S.S., and W. Hauter. 2001. "Preventing pathogenic food poisoning: Sanitation not irradiation," *Intl. J. of Health Services* 31:187-192.

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Geneva.<sup>3</sup> These ten positive studies compare to only 17 published *in vivo* studies that were reportedly negative for mutagenicity. Similarly, for published *in vitro* studies, five mutagenicity studies were positive and 8 were negative. **Overall, more than one-third of published studies indicate mutagenicity of irradiated food substances.** Further, several recent *in vitro* and unpublished *in vivo* studies from the respected Karlsruhe irradiation research facility in Germany have uncovered mutagenic effects in human and animal cells, and in lab animals.

Below are samples of quotes from studies where negative health effects from high-dose irradiation were observed (emphasis added):

- “*Freshly irradiated PRD diet fed to male mice of both strains caused an **increase in early deaths of offspring** of females mated to the males in week 7 and to a lesser extent in week 4.” (Study 1, below)*
- “*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.” (Study 2)*
- “*Feeding of mice (males and females) for two months before mating with 50% of the standard complete diet (solid cakes) irradiated with 5 Mrads of radiation provokes a **significant increase of pre-implantation embryonal deaths.**” (Study 3)*
- “*The children receiving freshly-irradiated wheat **developed polyploid cells and certain abnormal cells** in increasing numbers as the duration of feeding increased and showed a gradual reversal to basal level of nil after withdrawal of the irradiated wheat. In marked contrast, none of the children fed unirradiated diet developed any abnormal cells.” (Study 7)*

Safety of high-dose irradiation above 10 kGy has not been shown. An objective, dispassionate, review of the detailed information presented in this comment will lead to the firm conclusion that the CCFAC proposed revision to the Codex irradiation standard amounts to a serious - potentially scandalous - error in judgment based on a flawed report.

### **POSITIVE STUDIES MENTIONED BUT MISCHARACTERIZED IN THE 1999 FAO/IAEA/WHO REPORT**

The 1999 FAO/IAEA/WHO report incorrectly labeled each of the first four published irradiation studies summarized below as “*negative for high-dose irradiation effect, possible effect of nutrition or diet.*” This was a mischaracterization because each of these *in vivo* mammalian studies was plainly in fact “positive,” meaning that mutagenic effects were found associated with the irradiated food. In sum, the 1999 FAO/IAEA/WHO report fails to justify its

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<sup>3</sup> Mutagenicity is the capacity to cause gene-damage that may result in gene mutations, polyploidy, chromosome aberrations, and dominant lethal mutations. Anderson, D., et al. 1981. Irradiated laboratory animal diets - Dominant lethal studies in the mouse. *Mutation Research* 80:333-345.

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off-hand, non peer-reviewed, but crucial, suggestion that the positive results of these published studies - each conducted in respected laboratories - represented mere artifacts of the nutrition or diet in the experiments.

Studies 1 through 4 were cited frequently in the report and no indication exists of defects in their approaches or methodologies. The studies and discussion of the 1999 FAO/IAEA/WHO report's rationalizations for them are below:

**Study 1.** Anderson, D., M.J.L. Clapp, M.C.E. Hodge, and T.M. Weight. 1981. Irradiated laboratory animal diets - Dominant lethal studies in the mouse. *Mutation Research* 80:333-345.

### **Study 1 abstract:**<sup>4</sup>

In 4 separate dominant lethal experiments, groups of mice were fed laboratory diets (Oakes, 41B, PRD, BP nutrition rat and mouse maintenance diet No.1). The diets were either untreated (negative control diets) or irradiated at 1, 2.5, and 5 Mrad and were freshly irradiated, or stored.<sup>5</sup> The animals were fed their test diets for a period of 3 weeks prior to mating. Groups of mice given a single intraperitoneal injection of 200 mg cyclophosphamide per kg body weight served as the positive controls. Freshly irradiated PRD diet fed to male mice of both strains caused an increase in early deaths of offspring of females mated to the males in week 7 and to a lesser extent in week 4. The increase due to irradiation was small by comparison with that produced by the positive control compound. The responses for the other irradiated diets showed no significant increases in early deaths of offspring, although some values for Oakes diet were high. The effect of storage was examined with PRD and BPN diet on one occasion and produced conflicting results. Thus there was some evidence that irradiated PRD diet has weak mutagenic activity in the meiotic and/or pre-meiotic phase of the spermatogenic cycle, which appeared to be lessened on storage.

**Discussion of Study 1:** The 1999 FAO/IAEA/WHO report acknowledged the Anderson et al. study showed "*evidence of weakly mutagenic effect*" with one diet that was irradiated, yet it classified the study as "*negative for high-dose irradiation effect, possible effect of nutrition or diet*" (p. 117). However, no indication exists that the irradiated standard PRD laboratory diet that produced the mutagenic effect was otherwise deficient. Further, the unirradiated control PRD diet did not produce the mutagenic effect. Anderson et al. found irradiation of the diet produced the effect. The 1999 FAO/IAEA/WHO report's classification of the study as "negative" was unfounded.

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<sup>4</sup> Abstracts are virtually verbatim from the published articles, with minor edits for readability.

<sup>5</sup> Note that the unit measurement for irradiation doses was formerly Mrads and is currently kiloGrays (kGy). One Mrad is equivalent to 10 kGy.

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**Study 2.** Bugyaki, L., A.R. Deschreider, J. Moutschen, M. Moutschen-Dahmen, A. Thijs, and A. Lafontaine. 1968. Do irradiated foodstuffs have a radiomimetic effect? II. Trials with mice fed wheat meal irradiated at 5 Mrad. *Atompraxis* 14:112-118.

### **Study 2 abstract:**

Two groups of 10 male and 50 female mice were fed diet containing 50% wheat flour, which was either untreated or irradiated with 5Mrad of gamma radiation. 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. The procedure was repeated 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 were about 35% higher in the test group than in the controls. The life span of mice fed irradiated flour was slightly shorter than in the control mice.

### **Discussion of Study 2:**

A thorough discussion of the Bugyaki et al. study in a 1970 FAO/IAEA/WHO Expert Committee report highlighted it as a significant positive finding.<sup>6</sup> That earlier report, at pp. 28 and 29, stated:

*The Committee took cognizance of certain disturbing effects 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 demonstrable at the dose levels of practical importance.*

The 1999 FAO/IAEA/WHO report admitted that Bugyaki et al. showed "*chromosomal abnormalities in germ cells due to formation of peroxides and radicals*," but - without explanation - classified the study as "*negative for high-dose irradiation effect, possible effect of nutrition or diet*" (p. 118). That is plain inconsistency; the "peroxides and radicals" resulted

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<sup>6</sup> 1970 FAO/IAEA/WHO Technical Report #451. *Wholesomeness of Irradiated Food with Special Reference to Wheat, Potatoes and Onions*, WHO, Geneva.

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from the radiation (see Bugyaki et al., at p. 118: "...some of the changes produced by radiation – the free radicals for example – will disappear with time..." [translated from French]). Further, the same Expert Committee agreed 29 years earlier that Bugyaki et al. demonstrated "*certain disturbing effects*" of high-dose irradiation. That Committee did not discount the effects as artifacts of nutrition or diet, as the 1999 Committee did. The 1999 FAO/IAEA/WHO report's classification of this study as "negative" again lacks a rational foundation.

**Study 3.** Moutschen-Dahmen, M., Moutschen J., and L. Ehrenberg. 1970. Pre-implantation death of mouse eggs caused by irradiated food. *International Journal of Radiation Biology* 18:201-216.

### **Study 3 abstract:**

Feeding of mice (males and females) for two months before mating with 50% of the standard complete diet (solid cakes) irradiated with 5 Mrads of radiation provokes a significant increase of pre-implantation embryonal deaths, but no increase of post-implantation deaths. The pre-implantation deaths have probably to be interpreted as dominant lethal mutation associated with gross chromosomal aberrations, such as centromeric breaks repeatedly found to be induced by irradiated materials. Other interpretations, such as enhanced ageing, cannot be ruled out, however, on the basis of the present data. The investigation demonstrates disturbances in the fertilization processes as well as in development of eggs.

### **Discussion of Study 3:**

The 1999 FAO/IAEA/WHO report states the study showed "*increased pre-implantation embryonic deaths; not confirmed by cytological analysis*" and classified the study as "*negative for high-dose irradiation effect, possible effect of nutrition or diet*" (p. 115). The suggestion of an effect of nutrition or diet is unsupported. Further, the suggestion that the observed pre-implantation deaths were "not confirmed by cytological analysis" is a non sequitur. There was no question that the increased pre-implantation deaths occurred, and the study did not include any cytological examination.

**Study 4.** Johnston-Arthur T., M. Brena-Valle, K. Turanitz, R. Hruby, and G. Stehlik. 1975. Mutagenicity of irradiated food in the host mediated assay system. *Studia Biophysica, Berlin* 50:137-141.

### **Study 4 abstract:**

Groups of Swiss albino mice (SPF) fed with normal and gamma-irradiated food at doses of 0.75, 1.5, and 3.0 Mrad, were injected intraperitoneally with *Salmonella typhimurium* TA 1530 for the host mediated assay test of mutagenesis. The mutation frequency was calculated in terms of the number of mutant colonies per

unit number of surviving cells. The results indicate that there is a significant increase in mutation frequency induced by the 3 Mrad sterilized food. No difference was observed in the 0.75 Mrad dose when compared with the control.

**Discussion of Study 4:**

The 1999 FAO/IAEA/WHO report admits the study showed "*significant increase in the mutation frequency induced by the high-dose irradiated foods,*" but nevertheless classified the study as "*negative for high-dose irradiation effect, possible effect of nutrition or diet*" (p. 115). This is patently contradictory; the "negative" classification again lacks explanation.

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The following positive *in vitro* human cell study, and the companion positive *in vivo* mammal study discussed after it, resulted in a lengthy text rationalization in the 1999 FAO/IAEA/WHO report:

**Study 5 (*in vitro*).** Delincée, H., and B.L. Pool-Zobel. 1998. Genotoxic properties of 2-dodecylcyclobutanone, a compound formed on irradiation of food containing fat. *Radiation Physics and Chemistry* 52:39-42.

**Study 5 abstract:**

When food containing fat is treated by ionizing radiation, 2-dodecylcyclobutanone (2-DCB) is formed. To date there is no evidence of this compound in unirradiated food, that is, it is unique to irradiated foods. Therefore it cannot be considered inherent in food and it is advisable to determine whether it is toxic. Measurements of DNA damage in cells exposed to 2-DCB were carried out. *In vitro* experiments using rat and human colon cells indicate that 2-DCB in the concentration range of about 0.30 - 1.25 mg/ml induces DNA strand breaks in the cells. To what extent these *in vitro* findings are relevant for the *in vivo* human exposure situation needs to be further investigated.

**Study 6 (unpublished).** Delincée, H., B.L. Pool-Zobel, and G. Rechkemmer. 1998. Genotoxicity of 2-dodecylcyclobutanone. *Food Irradiation: Fifth German Conference*, Report BFE-R-99-01, Federal Nutrition Research Institute, Karlsruhe, Germany.

**Study 6 abstract:**

In the treatment of foods containing fat with ionizing radiation - for example, the irradiation of chicken or hamburger to kill pathogens such as *Salmonella spp.* or *E. coli* O157:H7 - a range of lipolytic digestion products are generated, among them the group of 2-alkylcyclobutanones. These compounds contain the same

number (n) of carbon atoms as their precursor fatty acids, whereby a hydrocarbon chain with n-4 carbon atoms is attached to ring position 2 of the cyclobutanone. In this way, 2-dodecylcyclobutanone (2-DCB) is generated from palmitic acid. Up to the present day, cyclobutanones have not been found in non-irradiated foods. Therefore, it is important to examine the toxic or genotoxic potential of cyclobutanones in the context of discussions about the safety of irradiated foods.

In this study, *in vivo* experiments were conducted on rats, which received two different doses of 2-DCB by way of pharyngeal probe. After 16 hours, colon cells were isolated from the rat and analyzed for DNA damage by means of the comet assay. No cytotoxic effects were detected in the trypan blue vitality test. When the "% tail intensity" or the "tail moment" was used in the comet assay for quantitative analysis, the values obtained with an experimental group that received a low concentration of 2-DCB (1.12 mg/kg body weight) were similar to those of the control group, which was administered 2% dimethyl sulfoxide. Slight but significant DNA damage was observed in the experimental group that received the higher concentration of 2-DCB (14.9 mg/kg body weight). Further studies are needed to clarify the relevance of these results to an evaluation of risk from the consumption of irradiated foods.

#### **Discussion of Studies 5 and 6:**

The 1999 FAO/IAEA/WHO report properly labeled Study 5 as demonstrating a “*possible effect of high-dose irradiation.*”<sup>7</sup> But, it rationalized this by saying the level of the lipid present in the experiment was three orders of magnitude greater than the normal lipid level in chicken meat.

In the discussion of Study 5 in the report text, a late note added in the manuscript proof by the WHO Secretariat states:

*In a subsequent in vivo study, [Study 6, here] as yet unpublished, the researchers claim to have found a small positive effect when six rats were administered an extremely high level of the synthetically-prepared 2-DCB. (Fn. 1, p. 124)*

But, Study 6 did not, in fact, use an “*extremely high level*” of 2-DCB as claimed in the WHO Secretariat’s proof note. The level of 2-DCB, according to the researchers, was carefully calibrated and multiplied by the appropriate toxicological safety factor, to determine the safety of chicken irradiated for shelf sterilization at 60 kGy, a level that would be allowed under the pending CCFAC proposal. Delincée et al. conclude that applying the standard toxicological safety factor of 100 below the “no-effect level” means that 2-DCB failed the standard safety test. The WHO Secretariat’s allegation that the level was “*extremely high*” begs the question

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<sup>7</sup> The 1999 FAO/IAEA/WHO report shows bias not only in its misclassification of certain studies, but also in the basic classifications it uses. See keys for Table 31, at p. 113, and Table 32, at p. 118. Negative studies are classified unqualifiedly as “negative for high-dose irradiation effect.” But, positive studies are classified conditionally, as “possible effect of high-dose irradiation” (emphasis added). This facially unequal treatment is not explained.

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of why an experienced irradiation researcher would use an incorrect level of the substance for toxicological testing?<sup>8</sup>

The significance of Studies 5 and 6 lies in their safety testing of a substance now known to be unique to irradiated foods. Indeed, one of the arguments formerly made in support of the safety of irradiated foods was this:

*The difficulty of detecting characteristic differences between irradiated and unirradiated foods is part of the evidence that irradiated foods are as safe as unirradiated foods.*<sup>9</sup>

Now researchers have identified a unique marker for irradiated foods, **and the marker happens to be genotoxic, causing DNA strandbreaks in human and rat cells, in both *in vitro* and *in vivo* testing.** In addition to his calls for further research in Study 6, Dr. Delincée has made the following points to an author of this comment (pers. comm. to Peter T. Jenkins):

*Since we would like to know whether in case of cyclobutanones these DNA strandbreaks have any significance, we concluded that further experiments are required. Thus, a large database with results from both in vitro and in vivo testing is needed, combined with the results of appropriately designed multilaboratory international validation studies.*

The recommended further tests have yet to be completed and published. However, a preliminary, unpublished, example of the ongoing studies by Dr. Delincée and his associates has produced further disturbing results. (Delincée, H., C. Soika, and E. Marchioni. 2001. "Genotoxicity of 2-alkylcyclobutanones, markers for an irradiation treatment in fat-containing food," *12<sup>th</sup> International Meeting on Radiation Processing, Conference Abstracts, 25-30 March 2001*, Avignon, France, pp. 148-149.) According to that recent study, further "*in vivo tests with rat colon cells are planned to supplement these results.*"

Based on the above, it seems inconceivable that the CCFAC rejected the basic recommendation of Delincée et al. that "*the results urge caution and should provide impetus for further studies*" (Study 6, above). The full Codex Commission should ensure that the needed further studies are completed and published in peer-reviewed journals before considering a proposal to allow higher irradiation levels. Proceeding otherwise is contrary to science.

### **Discussion of 2-DCB and Palmitic Acid:**

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<sup>8</sup> While the simulation of the 60 kGy concentration of 2-DCB, representing sterilized chicken, was clearly genotoxic in the 6 rats tested, the more alarming result may have been from the lower concentration of 2-DCB. According to the study, "The low concentration was meant to model radiation pasteurization (e.g., with 3 kGy)." This represents the level commonly used now when irradiating chicken. Even at that ordinary level, 2 of the 6 rats in the experiment indicated a genotoxic response. However, when the responses of all 6 rats' were combined, the difference was not statistically significant relative to the control group.

<sup>9</sup> Swallow, A.J. 1991. Wholesomeness and safety of irradiated foods. In M. Friedman, ed., *Nutritional and Toxicological Consequences of Food Processing*. Plenum Press, NY, at p. 17.



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Numerous studies conducted since 1990 have identified 2-DCB as a unique irradiation byproduct of palmitic acid at doses as low as 0.5 kGy.<sup>10</sup> These studies have identified 2-DCB in numerous types of food, including beef, pork, lamb, chicken, eggs, mangoes, papayas, cheese, and freshwater, seawater and anadromous fish.<sup>11, 12</sup> In fact, 2-DCB, which has never been found in any non-irradiated food,<sup>13</sup> is so readily identifiable as a unique irradiation byproduct of palmitic acid that it is commonly used as a marker for irradiated food — a byproduct that has been shown to persist in food up to 13 years.<sup>14</sup>

Palmitic acid is ubiquitous in foods, appearing in pronounced quantities in virtually all types of meat (including fish and shellfish), vegetables, fruit, grains, dairy products and vegetable oils.<sup>15</sup> Palmitic acid also appears in pronounced quantities in dozens of ready-to-eat foods, including sauces, pizzas, baked goods, snack foods and many other types of food.<sup>16</sup>

Because palmitic acid appears in an enormous variety of foods in varying quantities, the Codex Commission should refrain from considering the Proposed Draft Revision until an inventory of foods covered by the General Standard for Irradiated Foods is conducted, and the potential cytotoxicity and genotoxicity of 2-DCB in each class of food is thoroughly studied.

### **POSITIVE STUDIES IGNORED IN THE 1999 FAO/IAEA/WHO REPORT**

Studies 1 through 6 at least received mention in the 1999 FAO/IAEA/WHO report. But, it outright ignored the studies below, numbered 7 through 12, which found mutagenic effects in feeding experiments with human children, mice, rats, and monkeys. They were published in four reputable, peer-reviewed, scientific journals, including the American Journal of Clinical Nutrition.

**Study 7.** Bhaskaram, C., and G. Sadasivan. 1975. Effects of feeding irradiated wheat to malnourished children. *American J. of Clinical Nutrition* 28:130-135.

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<sup>10</sup> Stevenson, M.H. Identification of irradiated foods. *Food Technology*, 48: 141-144, 1994.

<sup>11</sup> Ibid.

<sup>12</sup> Stewart, E.M. et al. 2-alkylcyclobutanones as markers for the detection of irradiated mango, papaya, Camembert cheese and salmon meat. *Journal of the Science of Food and Agriculture*, 80: 121-130, 2000.

<sup>13</sup> Stevenson, op cit.

<sup>14</sup> Crone, A.V.J. et al. Detection of 2-dodecylcyclobutanone in radiation-sterilized chicken meat stored for several years. *International Journal of Food Science and Technology*, 27: 691-696, 1992.

<sup>15</sup> Chow, C.K. ed. *Fatty Acids in Foods and Their Health Implications*. New York: Marcel Dekker, 2000.

<sup>16</sup> Ibid.

**Study 7 abstract:**

Fifteen children suffering from severe protein-calorie malnutrition were divided into three groups of five each and received diets containing either unirradiated, freshly-irradiated, or stored irradiated wheat. All the children were hospitalized for a period of 6 weeks and leukocyte cultures were done initially and at intervals of 2 weeks. The children receiving freshly-irradiated wheat developed polyploid cells and certain abnormal cells in increasing numbers as the duration of feeding increased and showed a gradual reversal to basal level of nil after withdrawal of the irradiated wheat. In marked contrast, none of the children fed unirradiated diet developed any abnormal cells while the children fed stored irradiated wheat showed some polyploid and abnormal cells, but in significantly decreased numbers compared to the children fed freshly-irradiated wheat. Although the biological significance of polyploidy is not clear, its association with malignancy makes it imperative that the wholesomeness of irradiated wheat for human consumption be very carefully assessed.

**Study 8.** Vijayalaxmi. 1975. Cytogenetic studies in rats fed irradiated wheat. *Int. J. Radiat. Biol.* 7:283-285.

**Study 8 abstract:**

Thirty Wistar weanling rats were divided into three groups (each 5 M and 5 F). One was fed a diet including unirradiated wheat, the second the same diet with freshly-irradiated wheat (less than 20 days after irradiation), and the third the same diet with irradiated wheat stored for at least 12 weeks. The irradiation dose was 75 krads. Twelve weeks after feeding these diets, all the rats were killed and their bone-marrow processed for cytogenetic analysis. For each rat, 50 well-spread cells in metaphase were examined for aberrations and 500 cells were examined to determine the incidence of polyploid cells. There were no significant differences in the incidence of aberrations (breaks and deletions) among the 3 groups. However, the incidence of polyploid cells was significantly higher in the group fed freshly-irradiated wheat relative to the 2 other groups.

In a related second experiment, 42 Wistar weanling rats were divided into 7 groups (each 3 M and 3 F). They were fed freshly-irradiated wheat and killed at intervals of 1, 2, 3, 4, 6, 8 and 10 weeks. The bone marrow was processed for chromosomal analysis and for each rat 1,000 cells were examined to determine the incidence of polyploid cells. There was a progressive increase in the number of polyploid cells as the duration of feeding increased. The incidence of polyploid cells was significantly higher by the sixth week compared to those fed unirradiated wheat in the first experiment. These results suggest that during irradiation of wheat, a toxic substance or substances are formed, the concentration of which falls after 12 weeks storage.

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**Study 9.** Vijayalaxmi. 1976. Genetic effects of feeding irradiated wheat to mice. *Canadian Journal of Genetics and Cytology* 18:231-238

**Study 9 abstract:**

The effects of feeding irradiated (75 krads) wheat in mice on bone marrow and testis chromosomes, germ cell numbers and dominant lethal mutations were investigated. Feeding of freshly-irradiated wheat resulted in significantly increased incidence of polyploid cells in bone marrow, aneuploid cells in testis, reduction in number of spermatogonia of types A, B, and resting primary spermatocytes as well as a higher mutagenic index. Such a response was not observed when mice were fed stored irradiated wheat. Also there was no difference between the mice fed unirradiated wheat and stored irradiated wheat.

**Study 10.** Vijayalaxmi. 1978. Cytogenetic studies in monkeys fed irradiated wheat. *Toxicology* 9:181-4.

**Study 10 abstract:**

Twenty-one monkeys, *Macaca mulatta*, around 6-8 months old, were divided into 3 groups of 7 each (4 M and 3 F) and fed diets containing unirradiated wheat, freshly-irradiated wheat (at 75 krads; every 20 days a fresh batch of irradiated wheat was used), and stored irradiated wheat (after irradiation it was stored for a period of not less than 12 weeks before being used). Blood samples were collected initially and again 4 and 10 months after feeding the diets, so that each animal served as its own control. At the end of 10 months, the monkeys that received the freshly-irradiated wheat diet were switched to the diet containing unirradiated wheat while the animals on unirradiated wheat continued to receive the same diet. Blood samples were collected 2 and 7 months later. There were no observed differences among the 3 groups regarding the frequency of structural aberrations like breaks and deletions. However, after examining more than 150 cells in metaphase for each animal, an increased incidence of numerical aberrations was observed in the form of endoreduplicated cells (4n) in the group fed the freshly-irradiated wheat diet. No such changes were observed in the other groups. Following the replacement of freshly-irradiated wheat with unirradiated wheat, the incidence of endoreduplicated cells decreased.

**Study 11.** Vijayalaxmi and G. Sadasivan. 1975. Chromosomal aberrations in rats fed irradiated wheat. *Int. J. Radiat. Biol.* 27:135-142.

**Study 11 abstract:**

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The effects on chromosomal aberrations in bone-marrow cells of feeding irradiated wheat to well-fed and malnourished rats were investigated. Rats maintained on low levels of dietary protein (5 %) had a higher incidence of breaks and deletions in their bone-marrow chromosomes compared with rats maintained on adequate levels of protein (18 %), showing that the level of dietary protein *per se* can produce structural abnormalities. Feeding of irradiated wheat to rats was associated with an increase in the number of polyploid cells in the bone-marrow. The level of protein in the diet did not appear to influence polyploidy.

**Study 12.** Vijayalaxmi and K.V. Rao. 1976. Dominant lethal mutations in rats fed on irradiated wheat. *Int. J. Radiat. Biol.* 29:93-98.

### **Study 12 abstract:**

Thirty-two weanling male rats (Wistar) were divided into two groups of 16 each. One was fed a high protein and one a low protein diet. After 8 weeks, four males in each group were kept for mating to determine the effect of the different protein diets. The remaining 12 males in each group were divided into two subgroups of six each. One was fed on an irradiated wheat diet, the other on the same diet but unirradiated. At the end of 12 weeks, all males were used in mating experiments. The dominant lethal assay was used, in which all females were killed 13 days after the mid-week of their presumptive mating. A mutagenic index was calculated, based on the number of dead embryos in relation to the number of total implants. The other set of four male rats in each group was given the same high and low protein as well as wheat diets for the same duration and their testes were collected and analyzed to estimate the number of surviving germ cells.

Both well well-fed and malnourished rats had significantly higher mutagenic indexes when they were fed freshly-irradiated wheat diets compared to those fed unirradiated diets. Malnutrition *per se* had little effect on the rate of intrauterine deaths. The well-fed rats, whether eating irradiated or unirradiated diets, did not display significant differences in the number of germ cells. On the other hand, malnourished rats, when switched over to irradiated wheat diet, had significantly fewer germ cells than when given unirradiated wheat diet.

### **Discussion of Studies 7 through 12**

The complete absence of the above studies from the 1999 FAO/IAEA/WHO report gives it an appearance of incompleteness and bias. The report's authors should have considered an expert cancer researcher's commentary on these Indian National Institute of Nutrition (NIN) studies:

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*These experiments have been strongly criticised but have been confirmed by some independent experiments although not by others. Nevertheless, further well-conducted work would seem to be required if the question of the safety of freshly-irradiated wheat is to be resolved.<sup>17</sup>*

Most noted has been Study 7, the NIN study involving children. It is unfathomable for the 1999 FAO/IAEA/WHO report to have disregarded it, the only published, controlled, human study using freshly-irradiated food. The following Australian genotoxicity expert's testimony to a government commission examining food irradiation backed the study's validity:

*The [NIN children] study itself I guess could be criticized in some ways, although, given that it was carried out in 1975, when perhaps not so much was known about cytogenetics as today, it is a reasonable study. It is fairly small but they looked at quite a number of cells and the findings seemed reasonable.<sup>18</sup>*

The results of the NIN studies were factually supported, and the criticisms rebutted, by the researchers themselves in two later published defenses:

**Study 13.** Vijayalxmi and S.G. Srikantia. 1989. A review of the studies on the wholesomeness of irradiated wheat, conducted at the National Institute of Nutrition [NIN], India. *Radiation Phys. Chem.* 34:941-952.

### **Study 13 abstract:**

This paper recounts the earlier NIN study on feeding irradiated wheat to malnourished children. In this paper, the authors not only compared the results of the previous NIN study to results of several other mutagenicity studies, but also gave detailed answers to some of the criticisms made against the NIN study. They published more data from the children study and used supporting data from the other NIN animal studies (rat, mice, and monkey), some of which had not previously been published. They re-scored their slides of cells and had another cytogeneticist review them. This paper claims the reviews uphold the validity of the 1975 observations.

**Study 14.** Vijayalaxmi. 1999. Comparison of studies on the wholesomeness of irradiated wheat: A review. *Nutrition Research* 19:1113-1120.

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<sup>17</sup> Swallow, A.J. 1991. Wholesomeness and safety of irradiated foods. In M. Friedman, ed., *Nutritional and Toxicological Consequences of Food Processing*. Plenum Press, NY, at p. 20.

<sup>18</sup> Sutherland, G.R. 1988. *Official Hansard Report of the House of Representatives Standing Committee on Environment, Recreation and the Arts, Australia*. Evidence given to the Committee on the 26th Sept., 1988, Australian Govt. Publ. Serv., Canberra, p. 3842. Dr. Sutherland is Director of the Department of Cytogenetics and Molecular Genetics at the Women's and Children's Hospital, Adelaide, Australia. He pioneered investigation into fragile sites on chromosomes. He was President of the Human Genome Organization in 1996 and 1997, and a co-recipient of the 1998 Australia Prize.

**Study 14 abstract:**

This paper 24 years later again recounts the 1975 NIN study on the wholesomeness of irradiated wheat, and notes that similar experiments conducted by other investigators failed to replicate the NIN study conditions. A detailed examination of the published studies reveals several potentially critical differences among these investigations. Those differences are highlighted and the possibility of their contribution to the differences in the final results is discussed. The paper concludes that no prior studies exactly duplicated the experimental conditions and the diets used in the NIN study of malnourished children, and that the diet differences may have profoundly affected the outcomes. The paper concludes that the poor quality protein and the low availability of radical-scavenging micronutrients in the NIN diets may not have given the necessary protection against damage induced by the primary and secondary radiolytic products in the freshly-irradiated wheat. The author contends that in the absence of further investigations to resolve these controversies, the broad approvals given to food irradiation by FAO/IAEE/WHO expert groups appear scientifically unjustified.

Neither these follow-up papers nor the original NIN studies by Vijayalaxmi and others have ever been addressed in detail by critics in a published article. Again, the 1999 FAO/IAEE/WHO report authors omitted studies 7 through 14 altogether - despite compiling and citing 495 other irradiation studies - although they did see fit to include and cite the one study by Dr. Vijayalaxmi in which no mutagenic effect of irradiation was found.<sup>19</sup> Bias is apparent.

**THE NUMBER OF POSITIVE MUTAGENICITY STUDIES COMPARES FAVORABLY WITH THE NUMBER OF NEGATIVE STUDIES**

In sum, ten published *in vivo* mammal studies cited herein, including one human study, have found mutagenic effects from eating irradiated diets. In several instances these results have replicated and reinforced one another. No credible argument has surfaced that all ten studies were defective or spurious.

Remarkably, this total compares favorably with the number of published *in vivo* mammalian and human studies reportedly with negative findings for mutagenicity. The detailed 1999 FAO/IAEA/WHO report lists 17 *in vivo* studies of mammals published in scientific journals with negative findings for mutagenicity (after subtracting its misclassification of Studies 1 through 4, above.) (Table 1, below.<sup>20</sup>) For comparison, Table 1 also shows the proportion of published

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<sup>19</sup> Reference number 385 in the 1999 FAO/IAEE/WHO report; Vijayalaxmi. 1980. Sister chromatid exchanges in human peripheral blood lymphocytes grown in irradiated medium. *Intl. J. of Irradiation Biology* 37:581-583.

<sup>20</sup> The negative study numbers are the reportedly negative studies from the 1999 FAO/IAEA/WHO report, Table 32, pp. 114-118; studies are only included if they were published in a scientific journal (i.e., studies numbered: 387, 389, 390, 393, 398, 400, 401, 404, 405, 406, 407, 411, 412, 415, 416, 418, and 421); studies 388, 397, 413, and 414, are excluded because they were not journal-published. Studies 1 through 4 herein (respectively, studies numbered 408, 361, 402, and 407 in the 1999 FAO/IAEA/WHO report) are not considered negative studies in Table 1 because of misclassification; other than these four, the numbers of other negative studies are provided without any review of

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positive *in vitro* studies on mutagenic effects of irradiated foods in relation to the published negative *in vitro* studies, both as listed in the 1999 FAO/IAEA/WHO report.<sup>21</sup> The comparisons are very consistent; **more than one-third of both *in vivo* and *in vitro* studies are positive.**

**Table 1. Summary results of published studies on mutagenic effects of irradiated foods.**

	<b>Positive for mutagenicity</b>	<b>Negative for mutagenicity</b>
<b><i>In vivo</i> mammal (including human) studies</b>	10 (37 %)	17 (63 %)
<b><i>In vitro</i> studies</b>	5 (38 %)	8 (62 %)

Official suggestions in the past that positive mutagenicity findings have not been duplicated ring false, apparently resulting from bias. Vijayalaxmi and Srikantia, in Study 13, above, describe bias in the official posture in support of the safety of irradiation:

*It is difficult to escape from the feeling that all findings which are in favour of the wholesomeness of irradiated foods are readily accepted, while observations which raise doubts and question this stand are either viewed with suspicion, either covertly or overtly, or outrightly rejected.*

As shown above, the 1999 FAO/IAEA/WHO report either mischaracterizes or ignores the large percentage of positive studies, bolstering Vijayalaxmi and Srikantia’s contention. Perhaps the bias has resulted from a “groupthink” mentality among a small number of officials working in an esoteric field. These officials apparently believe they can rationalize, or “explain away,” the positive results, but they have failed to do so adequately. The small number of officials pushing

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the actual studies to determine whether they received proper classification. Non-mammal studies are excluded. The positive studies are Studies 1-4 and 7-12 discussed herein.

<sup>21</sup> The numbers of reportedly negative and positive *in vitro* studies are from the 1999 FAO/IAEA/WHO report, Table 31, pp. 112-113. The numbers are provided without any review of the actual studies to determine whether they received proper classification. Studies are only included if they were published in a scientific journal (i.e., negative studies numbered: 382, 384, 385, 386, 387, 389, 390, and 394; 388 is excluded because it was not journal-published; and positive studies numbered 383, 391, 392, 393, and 427). For the record, the positive studies are, in order: Niemand, J.G., et al. 1983. A study of the mutagenicity of irradiated sugar solutions: implications for the radiation preservation of subtropical fruits. *J. of Agric. and Food Chem.* 31:1016-1020; Shaw, M.W., and E. Hayes. 1966. Effects of irradiated sucrose on the chromosomes of human lymphocytes *in vitro*. *Nature* 211:1254-1256; Bradley, M.V., L.L. Hall, and S.J. Trebickock. 1968. Low pH of irradiated sucrose in induction of chromosome aberrations. *Nature* 217:1182-1183; Aiyar, A.S., and S. Rao. 1977. Studies on mutagenicity of irradiated solutions in *Salmonella typhimurium*. *Mutation Research* 48-17-28; and Delincée et al., Study 5 cited above.

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food irradiation safety needs to be compared with the large number of scientists who have stated its risks remain unresolved.

### **EXPERT CALLS FOR FURTHER RESEARCH**

Again, Delincée et al. of the respected Karlsruhe research facility, in Study 6, above, stated: “...*the results urge caution and should provide impetus for further studies.*” Numerous other experts have also called for, at least, more published research on irradiation safety.<sup>22</sup> Notably, 26 medical experts and many other prominent individuals recently endorsed a detailed warning in a health journal on the dangers of food irradiation.<sup>23</sup> The list of endorsers is impressive.<sup>24</sup> The Codex Alimentarius Commission cannot prudently ignore such explicit caution signals.

### **RECOMMENDATIONS TO STUDY RADIOLYTIC PRODUCTS IN HIGH-DOSE IRRADIATED FOOD HAVE NOT BEEN FOLLOWED**

The 1999 FAO/IAEA/WHO report did not follow the 1994 recommendation by the International Consultative Group on Food Irradiation (ICGFI) that “the literature be searched for identification and quantification of radiolytic products ... and that the presence of such products be evaluated for possible toxicological concern.”<sup>25</sup>

In Section 3 of the 1999 FAO/IAEA/WHO report, there is a discussion about the radiation chemistry of food irradiation, but little discussion about the identity of radiolytic products and essentially no discussion about the possible toxicity of such products.

Additionally, in the Conclusions section of the 1999 FAO/IAEA/WHO report, two significant statements are made without the benefit of supporting evidence:

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<sup>22</sup> See, e.g., Louria, D.B. 1993. Food irradiation: Perceptions of a qualified opponent. *Infectious Diseases in Clinical Practice* 2:313-316; Tritsch, G.L. 2000. Food irradiation. *Nutrition* 16:698-701; Steinberg, J., quoted in R. Papazian 1992. Food irradiation - A hot issue, *Harvard Health Letter*, vol. 17, no. 10, p. 3.

<sup>23</sup> Epstein, S.S., and W. Hauter, cited in fn. 2, above.

<sup>24</sup> Some examples of prominent MD and Ph.D. endorsers of the warning: Neal Barnard, President, Physicians Committee for Responsible Medicine; Donald Dahlsten, Professor and Associate Dean, Univ. of California, Berkeley; Robert Elder, Senior Microbiologist, Neogen Co.; Samuel Epstein, Emeritus Professor of Environmental Medicine, Univ. of Illinois School of Public Health, and Chairman of the Cancer Prevention Coalition; Jay M. Gould, Director, Radiation and Public Health Project; William Lijinsky, past Director of Chemical Carcinogenesis, Frederick Cancer Research Center; Donald Louria, Chairman, Department of Preventive Medicine, New Jersey Medical School; Vincente Navarro, Professor, The Johns Hopkins Univ. and Univ. of Pompeu Fabra, Spain; and Dr. Quentin Young, past President, American Public Health Association.

<sup>25</sup> International Consultative Group on Food Irradiation. *Review of Data on High Dose (10-70 kGy) Irradiation of Food: Report of a Consultation, Karlsruhe, 29 August - 2 September 1994*. Geneva: World Health Organization, 1995.



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- “Compounds found in irradiated model systems that are either far different in composition from the foods of interest or have been irradiated under extreme conditions do not validly reflect the chemistry (or toxicology of actual foods);” and
- “Virtually all of the radiolysis products found in high-dose irradiated foods to date are either naturally present in foods or produced in thermally processed foods.”

Concerns about radiolytic products and their possible toxicity date back many years. In 1977, the Joint FAO/IAEA/WHO Expert Committee recommended that, in order to “increase general knowledge about the consequences of food processing by irradiation,” work should be conducted into the “Further identification of radiolytic products and, where appropriate, determination of their toxicity.”<sup>26</sup>

Very little effort was made to follow up on this recommendation. In a 1981 report, the Joint FAO/IAEA/WHO Expert Committee offered little discussion of the identity of radiolytic products and essentially no discussion about the possible toxicity of such products. In the section “Aspects of Radiation Chemistry,” the committee cites the “unpublished observations” of several researchers and one study, which only examined the radiolytic products in irradiated beef.<sup>27</sup>

Accordingly, we strongly urge that the Codex Commission refrain from considering the Proposed Draft Revision until FAO, IAEA, ICGFI, WHO and any other appropriate agencies comply with these recommendations by fully investigating the identity, quantity and possible toxicity of radiolytic products. Sound science dictates that a full accounting and assessment of the radiolytic products in irradiated food would reduce the risks that irradiated food could pose to the population.

### **CCFAC OFFERED NO BASIS TO MAKE FOOD IRRADIATION STANDARDS OPTIONAL**

At its March meeting in The Hague, CCFAC provided no basis when it endorsed changing the word “shall” to “should” in nine instances within the text of the Proposed Draft Revision.

As a result of these changes, if the Codex Commission adopts the Proposed Draft Revision, irradiated food would no longer have to be:

- “of suitable quality,”
- in “acceptable hygienic condition,” or
- “handled ... according to good manufacturing practices.”

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<sup>26</sup> *Wholesomeness of Irradiated Food: Report of a Joint FAO/IAEA/WHO Expert Committee*. WHO Technical Report Series 604. Geneva: World Health Organization, 1977.

<sup>27</sup> *Wholesomeness of Irradiated Food: Report of a Joint FAO/IAEA/WHO Expert Committee*. WHO Technical Report Series 659. Geneva: World Health Organization, 1981.

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Additionally, food irradiation facilities would no longer have to:

- comply with “safety” and “good hygiene practices,”
- be staffed by “adequate, trained and competent personnel,”
- be licensed or inspected by government officials, or
- maintain certain records on radioactive activities.

Also, food irradiation would no longer have to be:

- carried out “commensurate with ... technological and public health purposes,” or
- conducted “in accordance with good radiation processing practice.”

The Codex Commission should refrain from considering the Proposed General Standard until CCFAC provides a basis for changing the word “shall” to “should,” including but not limited to a comprehensive analysis of how optional standards would affect the safety and wholesomeness of irradiated food, and on the operational safety of irradiation facilities.

## **CONCLUSION**

The 1999 FAO/IAEA/WHO report and the Codex CCFAC proposal whitewash a potentially serious and pervasive human health concern, made more serious by several pending proposals to irradiate a much larger portion of the human food supply.<sup>28</sup> Is it conceivable that the Codex Alimentarius Commission would approve higher doses of a food technology when the studies demonstrating mutagenic effects actually amount to more than one-third of all the published mutagenicity studies, both *in vitro* and *in vivo*? Would the Commission approve any other additive if such a large proportion of the published studies - including the only controlled human study using freshly-irradiated food - demonstrated a real potential to cause gene damage to consumers and their offspring? Merely asking these questions illustrates the potentially scandalous nature of the CCFAC proposal.

In sum, the numerous positive *in vitro* and *in vivo* studies, together with the warnings from competent irradiation and mutagenicity experts, indicate a lack of adequate proof of safety for food irradiation. The known health risks would magnify at higher irradiation levels. Raising the allowable absorbed dose above the existing 10 kGy limit would be imprudent and potentially unsafe at this time. The Center for Food Safety and Public Citizen strongly urge the Codex Commission to:

- **reject the bracketed CCFAC proposal to eliminate the existing 10 kGy limit,**
- **refer the matter back to CCFAC with requests for it to: 1) seek corrections to the misclassification and omission of positive studies in the 1999**

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<sup>28</sup> See, for example, food industry petitions to the Food and Drug Administration in the United States Federal Register (FR) for permission to irradiate the following products: “ready-to-eat” meat, poultry, and other products, 65 FR No. 3, p. 493; molluscan shellfish, 64 FR No. 201, p. 56351; and crustaceans, 66 FR No. 25, pp. 9086-9087.

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**FAO/IAEA/WHO report; 2) review the 10 kGy absorbed dose limit to determine whether it is currently too high to adequately protect public health based on the best available scientific information, including continuation of research into the potential toxicity of 2-DCB and other cyclobutanones, and**

- **refer the matter back to CCFAC with a request to provide a basis for changing the word “shall” to “should” in the Proposed Draft Revision, and to conduct a comprehensive analysis of the potential affects on the safety and wholesomeness of irradiated food, and on the operational safety of irradiation facilities.**

Thank you for your attention to this comment. For further discussion about the issues herein, and to request copies of the studies cited, please contact Mark Worth of Public Citizen at [mworth@citizen.org](mailto:mworth@citizen.org).

Sincerely,

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