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May 12, 2003

Ronald Burke
Bureau of Regulatory, International and Interagency Affairs
Health Canada
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Ottawa, Canada K1A 0L2

Re: Health Canada's Proposed Amendments to Division 26 of the *Food and Drug Regulations* to Extend the Use of Irradiation to Mangoes; Fresh, Prepared, Dried and Frozen Shrimp and Prawns; and Fresh and Frozen Poultry and Ground Beef

Dear Mr. Burke:

The Council of Canadians, the Sierra Club of Canada, the Polaris Institute and Public Citizen are pleased to submit this public comment on Health Canada's proposed amendments to Division 26 of the *Food and Drug Regulations* to extend the use of irradiation to mangoes; fresh, prepared, dried and frozen shrimp and prawns; and fresh and frozen poultry and ground beef, as published in the *Canada Gazette* Part 1, Vol. 136, No. 47, pp. 3502-3510, Nov. 23, 2002 (hereafter "Proposal").

We are submitting this comment to supplement our comment of March 21. Claudette Dalpé of the Bureau of Regulatory, International and Interagency Affairs stated to us that Health Canada would accept our additional comments beyond the original deadline of March 21. Our earlier comment addressed irradiated mangoes, poultry, and shrimp and prawns. This comment addresses irradiated ground beef and the issue of 2-alkylcyclobutanones (2-ACBs).

Ground Beef

Health Canada's evaluation of the safety and wholesomeness of irradiated ground beef is flawed and inadequate. Key research into the chemical changes that beef undergoes when it is irradiated, and the toxicity of irradiated beef is lacking. Further, a vast majority of the research cited in the toxicology section of the evaluation does not address toxicity issues.

Composition
Health Canada's "Composition" section (B.d.iv.) omits substantial evidence that toxic chemicals are formed in irradiated beef.

In the FASEB study cited in the evaluation,[1] 62 of the 65 chemicals found in raw, irradiated beef were detected at levels higher than the levels detected in raw, frozen, non-irradiated beef. Of these 62 chemicals, 53 were not detected in non-irradiated beef whatsoever – thus making them unique to raw beef. Among these 62 chemicals are five chemicals known or suspected to cause cancer or birth defects – benzene, ethanol, hexane, methyl ethyl ketone, and toluene; and five chemicals that may harm the central nervous system – acetone, ethyl mercaptan, heptane, nonane, and pentane.

Of the 36 chemicals found in cooked samples of irradiated and non-irradiated beef, 30 were detected in irradiated beef at levels higher than the levels detected in non-irradiated beef. Of these 30 chemicals, 16 were not detected in non-irradiated beef whatsoever – thus making them unique to cooked beef. Among these 30 chemicals are the same five chemicals known or suspected to cause cancer or birth defects referenced above, and the same five chemicals that may harm the central nervous system referenced above.

Apart from benzene and toluene, Health Canada conducted no evaluation of the potential harm that could be posed by other toxic chemicals formed in irradiated beef. Clearly, such an analysis is essential in assessing the safety of irradiated beef.

In addition, Health Canada's discussion of the presence of 2-alkylcyclobutanones in the ground beef evaluation – one paragraph with one citation – is wholly inadequate. Assuming that the agency addresses this issue in the separate evaluation on 2-alkylcyclobutanones, our comments below are directed at this separate evaluation.

Plainly, it would be unwise for Health Canada to move ahead with the Proposal lacking a more thorough review of the chemical byproducts of irradiation and their potential hazards to human health.

Toxicology

Health Canada's "Toxicological Studies" section (B.g.) is inadequate and omits substantial evidence that irradiated beef may be hazardous to human health.

Of the 50 studies cited in the evaluation, only 4 appear to be specifically related to the toxicity of irradiated beef. With more than 1,000 animal feeding studies having been conducted on irradiated foods over the past 50 years, we are frankly at a loss to explain the paucity of toxicology studies, and the presence in the bibliography of studies concerning such subjects as cholesterol oxides in Indian ghee, the carcinogenicity of steroids, and prostate secretions. Such studies seem wholly irrelevant to the question of the toxicity of irradiated ground beef.

Of the four studies that specifically relate to the toxicity of irradiated beef, two are scientifically inadequate, one was conducted on fruit flies[2] (a useful though a far from definitive bellwether of toxicity) and one was not published in a peer-reviewed journal.[3] In one of the inadequate studies, the group sizes were only 2 to 3 females; and only summaries were presented, without complete data. Among the data presented, it should be noted that the percentage of pups weaned from female dogs fed irradiated beef was lower than dogs fed non-irradiated food.[4] In the other inadequate study, the group size of dogs was only 2 males and 2 females.[5]

The evaluation omits three irradiated beef feeding studies that produced severe adverse health effects in animals:

> • "A considerable number of the second litter of the experimental group [of rats] died... Symptoms observed were marked edema of the face, ruffled hair coat, general incoordination, spastic hopping gait, and sometimes complete loss of movement with dragging of the hind quarters. Those pups most severely affected often became completely prostrated a short time before death... In no case were these symptoms noted in the control group... The probability [is that the pups] were suffering from the characteristic muscular dystrophy syndrome (commonly referred to as nutritional muscular dystrophy) known to result from a marginal vitamin E intake."[6]

> • "A significant number of rats consuming irradiated beef died from internal hemorrhage within 46 days, the first death of a male rat coming on the 11th day of feeding. This rat became sluggish on the 8th day of the regimen and started refusing food. He continued [to be] morbid during the next two days, did not eat any food, lost weight and appeared anemic. He was found dead on the 11th day. Post-mortem examination showed hemothorax, the blood had not clotted; there was bleeding also in the epididymis."[7]

> • "Hemorrhagic death had occurred in all males fed irradiated diets by day 34... There is evidence to suggest that inefficient absorption of vitamins, i.e. vitamin K, from the intestinal tract may contribute to a deficiency state."[8]

Additionally, recent research tested the levels of various fats and fatty acids in irradiated ground beef. The key finding is that irradiation at room temperature approximately doubled the prevalence of *trans* fatty acids, from 4.6 percent in unirradiated samples to 8.5 percent in samples irradiated at 4.5 kGy, [9] the maximum allowable dose for ground beef under Health Canada's Proposal.

In a crucial report on *trans* fatty acids issued last year by the National Academies of Sciences' Institute of Medicine, the coronary heart disease risks presented by these substances, which, again, are doubled in quantity when ground beef is irradiated, are as follows (emphasis added):

> Similar to saturated fatty acids, there is a positive linear trend between *trans* fatty acid intake and LDL cholesterol concentrations. Some evidence also suggests that trans fatty acids result in lower HDL cholesterol concentrations. Hence, the net result is a higher total cholesterol (or LDL cholesterol):HDL cholesterol ratio. This finding, combined with data from prospective cohort studies, has lead to the concern that dietary *trans* fatty acids are more deleterious with respect to coronary heart disease than saturated fatty acids. [10]

> Summary - There is a positive linear trend between *trans* fatty acid intake and total and LDL cholesterol concentration, and therefore increased risk of CHD, thus **suggesting a Tolerable Upper Intake Level (UL) of zero**. Because trans fatty acids are unavoidable in ordinary diets, achieving such a UL would require

> extraordinary changes in patterns of dietary intake. Such extraordinary adjustments may introduce other undesirable effects (e.g., elimination of foods, such as dairy products and meats, that contain trans fatty acids may result in inadequate intakes of protein and certain micronutrients) and unknown and unquantifiable health risks may be introduced by any extreme adjustments in dietary pattern. For these reasons, no UL is proposed. **Nevertheless, it is recommended that *trans* fatty acid consumption be as low as possible while consuming a nutritionally adequate diet.**

As indicated the evidence suggests a Tolerable Upper Intake Level of **zero**. In any event, *trans* fatty acid consumption should be minimized. This Institute of Medicine recommendation directly contrasts with the Health Canada Proposal to allow irradiation of ground beef. At a minimum, information regarding possible *trans* fat increases in ground beef must be obtained by Health Canada before deciding on the Proposal, and the agency must consider the cumulative macronutritional effect of Canadian citizens eating such foods in a prospective heavily irradiated diet.

The list of other documented non-cholesterol and non-CHD related health problems associated with *trans* fat is surely well-known to Health Canada. *Trans* fat:[11]

• lowers the amount of cream (volume) in milk from lactating females in all species studied, including humans, thus lowering the overall quality available to the infant;
• correlates to low birth weight in human infants;
• increases blood insulin levels in humans in response to glucose load, increasing risk for diabetes;
• affects immune response by lowering efficiency of B cell response and increasing proliferation of T cells;
• decreases levels of testosterone in male animals, increases level of abnormal sperm, and interferes with gestation in females;
• decreases the response of the red blood cell to insulin, thus having a potentially undesirable effect in diabetics;
• inhibits the function of membrane-related enzymes such as the delta-6 desaturase, resulting in decreased conversion of, e.g., linoleic acid to arachidonic acid;
• causes adverse alterations in the activities of the important enzyme system that metabolizes chemical carcinogens and drugs (medications), i.e., the mixed function oxidase cytochromes P-448/450;
• causes alterations in physiological properties of biological membranes including measurements of membrane transport and membrane fluidity;
• causes alterations in adipose cell size, cell number, lipid class, and fatty acid composition;
• adversely interacts with conversion of plant omega-3 fatty acids to elongated omega-3 tissue fatty acids;
• escalates adverse effects of essential fatty acid deficiency; and
• increases peroxisomal activity (potentiates free-radical formation).

To summarize the apparent risks of eating irradiated ground beef that is, for example, grilled are considerable:

• Red meat consumption is a well-known risk factor for a myriad of health problems.
• Flame grilling coats the beef with polycyclic aromatic hydrocarbons, which are known carcinogens.

• Grilling meat creates heterocyclic amines, which are mutagens and carcinogens associated with both respiratory tract cancers (from the smoke) and colon cancer.
• Irradiated beef contains the unique radiolytic products, 2-alkylcylcobutanones, which are genotoxic in concentration and act as colon tumor promoters if consumed together with known colon carcinogens (as are present in this case).
• The doubling of *trans* fat in irradiated compared to non-irradiated ground beef increases the risks of coronary heart disease.
• The *trans* fat increase also increases risks of a variety of other health problems, listed above.

With such an array of associated risks, it appears that irradiated ground beef should be declared unsafe and unwholesome.

Plainly, Health Canada has overlooked a substantial amount of evidence suggesting that irradiated ground beef is not safe for human consumption. The agency should not move ahead with the Proposal until a complete review of the data pertaining to the safety and wholesomeness of irradiated ground beef is conducted.

2-Dodecylcyclobutanone and other Alkylcyclobutanones

Health Canada's evaluation of the toxicity of 2-ACBs is flawed and inadequate. The assessment does not include a large amount of evidence indicating that 2-ACBs have carcinogenic-related, cytotoxic and genotoxic properties.

Several studies have been conducted since 1998, most notably a three-year study on various 2-ACBs formed in irradiated ground beef funded by the European Union. The study was conducted by scientists from several respected institutions, including Germany's Federal Research Center for Nutrition in Karlsruhe and Université Louis Pasteur in Strasbourg. The study, released to the public earlier this year, made these findings:

• "2-ACBs have cytotoxic and genotoxic properties under certain experimental conditions employing the human colon tumor cell lines."

• "All of the 2-ACBs were shown to induce oxidative DNA damage."

• "In *Salmonella typhimurium* bacteria, several 2-ACBs (2-tDCB, 2-dDCB and 2-DCB) exhibited clear cytotoxic effects."

• "It was shown that a very small amount of 2-ACBs can be recovered from fatty tissue, while a similar small amount is excreted in feces. These results indicate that 2-ACBs are largely metabolized or possibly stored in other parts of the body. Therefore, further studies are absolutely necessary in order to elucidate the metabolism of 2-ACBs."[12]

• "Using an experimental colon carcinogenesis model in rats, 2-ACBs, when tested at a high concentration, potentiate the effect of an inducing carcinogen on the long term. This was revealed by the increase of colonic preneoplastic lesions and the development of a higher number of colon tumours

with larger size, as compared to control animals, which were not exposed to 2-ACBs. This suggests that, in this model experiment, 2-ACBs, while they do not induce carcinogenesis *per se*, rather promote the colonic carcinogenesis process." [13]

The scientists made substantial recommendations and issued cautions:

> "[S]ince our results point to toxic, genotoxic and even tumor-promoting activity of several 2-ACBs, we consider it necessary that further research, including confirmation of our results by other laboratories, be conducted to permit an assessment of the possible risks associated with consumption of irradiated, fat-containing foods… Further research is required to precisely determine exposure to these substances, the precise dose-response relationship, and in particular the kinetics and metabolism of 2-ACBs in the living organism. All of this research is necessary to gain insight into the mechanisms of the toxic effects. Numerous questions still remain to be answered, and much research must still be done, before an informed risk assessment can be conducted… Caution should be exercised before any risk to consumers by exposure to these compounds is denied." [14]

> "[W]e feel that our new data…raise some doubts about or at least suggest that caution should be exercised before any risk to consumers by exposure to these chemicals is denied. At present, knowledge about the potential toxicity of 2-ACBs (including possible metabolites) and their toxic properties is very limited. Since these compounds are uniquely formed by irradiation and are not inherent in food, in our opinion, complementary studies are needed to make a qualified risk assessment. It needs to be shown that despite the presence of potentially cyto- and genotoxic radiation-induced agents, the consumption of irradiated fat-containing food is safe for consumers. Food safety continuously needs to be re-assessed according to the latest state of scientific knowledge…This re-assessment should also be valid for irradiated foods."[15]

<u>Plainly, it would be unwise for Health Canada to move ahead with the Proposal until the agency thoroughly analyzes the EU study and makes a determination as to whether, based on the study's findings, ground beef irradiated under the conditions prescribed in the Proposal poses human health hazards.</u>

Numerous other warnings and calls for additional research have been issued recently by scientists who discovered the toxic properties of 2-ACBs:

• "[M]ore experiments than these preliminary ones are required."[16]

• "[F]urther clarification is needed to determine whether these results are relevant to the safety of irradiated foods. The results urge caution, and should provide impetus for further studies."[17]

• "Further studies are progressing to elucidate the relevancy of these experiments for the actual human exposure to cyclobutanones by consuming irradiated fat-containing food."[18]

• "Since we would like to know whether in the case of cyclobutanones these DNA strandbreaks have any significance, we concluded that further experiments are required."[19]

• "In our opinion further investigations, including confirmation of our results by other laboratories, will help to elucidate a possible risk associated with the consumption of irradiated fat-containing foods."[20]

William Au, a genetic toxicologist at the University of Texas' Department of Preventative Medicine, made these remarks to the European Commission's Scientific Committee on Food, which dismissed the EU-funded study's findings of toxic properties of 2-ACBs:

> In the report from the committee, the admission of 2-ACBs as a promoter for colon cancer in rat is significant. A promoting agent does not induce cancer by itself but can significantly enhance the cancer inducing activities of certain carcinogens. As a result, tumors can be developed much earlier than expected and more tumor nodules (e.g. in the colon) can be detected. Since colon cancer is already a serious health problem in the population, the promoter activities of 2-ACBs can pose a serious health threat to the population. A major risk factor for colon cancer in humans is the consumption of food with high fat content, with charbroiled component, with high content of aromatic amine. Therefore, consumption of irradiated food that contains colon tumor promoters can significantly enhance the existing health problem. As we know, the higher the irradiation dose, the more 2-ACBs are generated, thus causing more concerns. The report clearly indicated that no standard animal feeding tests were carried out with the 2-ACB. In addition, the tumor promoter activities should be more systematically investigated. The report also indicates that the genotoxic activities of 2-ACBs have not been tested in other standard *in vitro* assays: gene mutation and chromosome aberrations in mammalian cells. The latter investigations are needed in addition to the standard bacterial mutation assay because the mammalian cell assay may pick up genotoxic activities of chemicals that are missed by the bacterial assay.[21]

Plainly, it would be unwise for Health Canada to move ahead with the Proposal when world's leading 2-ACB scientists have urged caution and called for more experiments into the toxic properties of these chemicals.

Since 1990, 2-ACBs have been detected in most of the foods covered by the Proposal. The chemicals are so prevalent that they are often used to determine whether fat-containing foods have been irradiated. The EU and England have adopted the detection of 2-ACBs as their official standard. 2-ACBs have been shown to persist for as long as 13 years,[22] and to withstand cooking,[23] and storage in air, in a vacuum, or in carbon dioxide.[24]

Ground Beef

• In a 2001 study, 2-ACBs were detected in frozen ground beef patties irradiated at 1.3, 2.7, 4.5 and 7.2 kGy. "Even after 9 months of frozen storage (-18ºC), irradiated hamburgers could be identified."[25]

• In a 2002 study, 2-dDCB and 2-tDeCB were detected in irradiated ground beef patties irradiated at doses of 0.5, 1.0, 2.5, 5.0 and 7.0 kGy. 2-dDCB was detected at all doses; 2-tDeCB was detected at the two higher doses. At each individual dose, the concentration of 2-dDCB was 33 percent to 100 percent higher in ground beef patties irradiated with electron beams than patties irradiated with gamma rays.[26]

• In a 1994 study, 2-dDCB and 2-tDCB were detected in beef irradiated at 1 and 5 kGy. Concluded Stevenson: "Since most foods contain at least some fat, [this detection] method should be applicable to a wide range of foods... 2-dDCB has never been detected in any unirradiated or microbiologically spoiled samples, and has always been found in irradiated samples even at doses as low as 0.5 kGy."[27]

• In the Burnouf study of 2001, 2-DCB, 2-dDCB, 2-tDCB, 2-dDeCB and 2-tDeCB were all detected in irradiated ground beef.[28]

• In a 2001 study, "significant levels" of 2-dDCB and tDCB were detected in minced beef irradiated at 2, 4, 6 8 kGy.[29]

Poultry

• In a 1992 study, it was discovered that the 2-dDCB concentration in irradiated chicken increased in proportion to the radiation dose (ranging from 10-60 kGy). Also detected was 2-tDCB, an irradiation by-product of stearic acid. Additionally, 2-dDCB was discovered in chicken irradiated 13 years earlier by another group of scientists, "making it useful as a qualitative marker on long-term storage."[30]

• In a 1990 study, gas chromatography-mass spectrometry was used to find 2-dDCB in minced chicken irradiated at 5 kGy. They reported that 2-dDCB could be detected 20 days after irradiation. Based on the ease with which this chemical was detected, they concluded: "Our technique shows that 2-dDCB is a potential post-irradiation marker for minced chicken meat and possibly for other products."[31]

• In a 1992 study, it was reported that 2-dDCB levels in irradiated chicken fell only slightly after 18 days of storage. Additionally, the chemical was detected in chicken irradiated at 1 kGy. Stevenson also found 2-tDCB in irradiated chicken. "The compounds," Stevenson wrote, "were not detected in the unirradiated samples."[32]

• In a 1993 study, it was found again that 2-dDCB levels in irradiated chicken increased in proportion to radiation dose (ranging from 1-10 kGy). They also reported, for the first time, that cooking either before or after irradiation did not remove the 2-dDCB. It was also reported that storing irradiated chicken for 21 days in air, in a vacuum or in carbon dioxide did not result in significant reductions of 2-dDCB. "The method has potential for the estimation of irradiation dose. [C]yclobutanones ... are likely to have potential for the identification of a range of foods of varying fat and fatty acid composition."[33]

• In a 1995 study, researchers at Saarland University in Saarbrücken, Germany detected a variety of cyclobutanones in duck irradiated at 2.5 kGy.[34]

Mangoes

• In a 2000 study, researchers at Queen's University of Belfast detected 2-dDCB, 2-tDCB and tDeCB in irradiated mangoes. In irradiated mangoes, 2-tDCB was "identified as the main marker," and it was

detected after 14 days of storage at doses as low as 0.1 kGy. As in several previous experiments, the cyclobutanones were formed in proportion to radiation dose.[35]

The Proposal does not thoroughly address the 2-ACB content of irradiated beef and poultry, despite the presence of numerous published studies. And, the Proposal does not address the 2-ACB content of irradiated mangoes, despite the presence of a published study.

Shrimp and Prawns

Further, the Proposal does not address the 2-ACB content of irradiated shrimp. Though published data on the detection of 2-ACBs in irradiated shrimp could not be located, it is expected that the chemicals would be formed in shrimp upon irradiation, due to the presence of the fatty acid precursors of 2-ACBs.

Table 1 details the fatty acid content in foods covered by the Proposal. As can be seen, the fatty acid precursors to 2-ACBs are present in all of the foods.

Table 1. Fatty acid content of irradiated foods covered by the Proposal.[36]

*Type of fatty acid**	*Myristic*	*Palmitic*	*Stearic*	*Palmitoleic*	*Oleic*	*Total*
Mangoes	*0.01*	*0.05*	*0.01*	*0.05*	*0.05*	*0.17*
Poultry						
Chicken	*0.09*	*2.33*	*0.63*	*0.60*	*3.74*	*7.39*
Turkey	*0.05*	*1.33*	*0.43*	*0.48*	*2.27*	*4.56*
Duck	*0.25*	*9.58*	*3.20*	*1.54*	*16.73*	*31.30*
Goose	*0.17*	*6.95*	*2.33*	*0.98*	*16.68*	*27.11*
Pheasant	*0.09*	*2.21*	*0.65*	*0.81*	*3.08*	*6.84*
Ground Beef	*0.17*	*1.42*	*0.24*	*0.74*	*2.40*	*4.97*
Shrimp	*0.02*	*0.14*	*0.10*	*0.05*	*0.11*	*0.42*

** Measured in grams/100 grams*

Table 2 details the effects of 2-ACBs found in irradiated ground beef, as reported in the EU study. All of the 2-ACBs that yielded adverse effects have either been detected in irradiated foods covered by the Proposal, or derive from fatty acids found in these foods. Two of the 2-ACBs – 2-tDCB and 2-tDeCB – were shown to promote the carcinogenesis process in rats.

Table 2. Findings of the EU study of the effects of 2-ACBs.

Type of 2-ACB / *Fatty acid precursor*	*2-DCB* / *Myristic*	*2-dDCB* / *Palmitic*	*2-tDCB* / *Stearic*	*2-dDeCB* / *Palmitoleic*	*2-tDeCB* / *Oleic*
Found in ground beef	*X*	*X*	*X*	*X*	*X*
Cyto- and genotoxic damage to human cells		*X*	*X*		*X*
Cytotoxic and oxidative DNA damage to human cells	*X*	*X*	*X*		*X*
Cytotoxic damage to bacteria	*X*	*X*	*X*		
Colon cancer promoter in rats			*X*		*X*
Stored in adipose tissue and present in rat feces			*X*		*X*

Though 2-ACBs have been detected in irradiated mangoes, and though the fatty acid precursors to 2-ACBs are present in shrimp, the Proposal does not address the potential health effects of eating these foods.

More generally, the Proposal does not explore the fatty acid content of foods covered by the Proposal. Thus, the types of 2-ACBs that could be formed in these foods, the levels of these 2-ACBs and, ultimately, the potential health effects of these 2-ACBs are not known.

Plainly, it would unwise for Health Canada to move ahead with the Proposal until the agency thoroughly studies the fatty acid content in foods covered by the Proposal, and the presence, longevity and potential health effects of 2-ACBs. Further, because analyses of mangoes and shrimp are not presented, the Proposal should not proceed until this work is done.

Finally, the list of studies that Health Canada considered in its evaluation of 2-ACBs is highly inadequate and primarily includes studies that relate little or in no way to the toxic qualities of these chemicals. Of the 55 studies listed, 7 appear to relate to 2-ACBs.

Attached is a 2-ACB bibliography that includes 61 studies and other documents that relate directly to the toxicity, detection and other issues concerning these chemicals. It would be highly unwise – if not negligent – for Health Canada to proceed with the Proposal without obtaining all available documents related to 2-ACBs.

Plainly, given the potential adverse health effects of 2-ACBs – namely the recent findings of their carcinogenic, genotoxic and cytotoxic properties – it would be highly unwise for Health Canada to proceed with the Proposal until the agency obtains all available research on 2-ACBs and conducts a thorough risk assessment.

Notes

[1] "Evaluation of the health aspects of certain compounds found in irradiated beef." Life Sciences Research Office, Federation of American Societies for Experimental Biology, Bethesda, Maryland. Prepared for U.S. Army Medical Research and Development Command, Department of the Army, Washington, D.C. DAMD-17-76-C-6055. August 1977. Supplements I and II, March 1979.

[2] Mittler, S. "Failure of irradiated beef and ham to induce genetic aberrations in *Drosophila*." *International Journal of Radiation Biology*, 35:583-588, 1979.

[3] Raica, N. and Howie, D.L. "Review of the U.S. Army wholesomeness of irradiated food program (1955-1966)." *Proceedings*, International Symposium on Food Irradiation; Karlsruhe, Germany, IAEA SM-73/5, pp.119-135, 1966.

[4] McCay, C.M. and Rumsey, G.L. "Effect of irradiated meat upon growrth and reproduction of dogs." *Federation Proceedings*, 19:1027-1030, 1960.

[5] Radomski, J.L. et al. "Chronic Toxicity studies on irradiated beef stew and evaporated milk." *Toxicology and Applied Pharmacology*, 7:113-121, 1965.

[6] Poling, C.E. et al. "Growth, reproduction, survival and histopathology of rats fed beef irradiated with electrons." *Food Res*, 20:193-214, 1955.

[7] Metta, V.C. et al. "Vitamin K deficiency in rats induced by feeding of irradiated beef." *Journal of Nutrition*, 69:18-21, 1959. (Co-sponsored by the Surgeon General of the U.S. Army)

[8] Mellette, S.J. and Leone, L.A. "Influence of age, sex, strain of rat and fat soluble vitamins on hemorrhagic syndromes in rats fed irradiated beef." *Federation Proceedings*, 19:1045-1048, 1960. (Co-sponsored by the Surgeon General of the U.S. Army)

[9] Brito, M.S. et al. "Effects of irradiation on *trans* fatty acids formation in ground beef." *Radiation Physics and Chemistry* 63:337-340, 2002

[10] Letter Report on Dietary Reference Intakes for *Trans* Fatty Acids. National Academies of Sciences, Institute of Medicine, Food and Nutrition Borad, Panel on Macronutrients, 2002. <www.iom.edu/iom/iomhome.nsf/Wfiles/TransFattyAcids/$file/TransFattyAcids.pdf>

[11] Health problems list from Trans Fatty Acid Fact Sheet <www.enig.com/0001t1b.html>. Mary G. Enig, Ph.D., F.A.C.N., Director, Nutritional Sciences Division, Enig Associates, Inc., Silver Spring, MD.

[12] D. Burnouf, H. Delincée, A. Hartwig, E. Marchioni, M. Miesch, F. Raul, D. Werner. Etude toxicologique transfrontalière destinée à évaluer le risque encouru lors de la consommation d'aliments gras ionisés - Toxikologische Untersuchung zur Risikobewertung beim Verzehr von bestrahlten fetthaltigen Lebensmitteln – Eine französisch-deutsche Studie im Grenzraum Oberrhein, Rapport final d'étude Interreg II, projet N° 3.171. BFE-R--02-02, Federal Research Centre for Nutrition, Karlsruhe, Germany, 2002.

[13] "Information about the potential toxicity of 2-alkylcyclobutanones, a group of substances exclusively formed upon irradiation of food containing fat." International Consultative Group on Food Irradiation, Dec. 2001.

[14] Burnouf et al 2001, Op. cit.

[15] Burnouf, D. et al. "Comment on a statement of the SCF [European Commission's Scientific Committee on Food] on a report on 2-alkylcyclobutanones." July 2002.

[16] Delincée, H. and Pool-Zobel, B. "Genotoxic properties of 2-dodecylcyclobutanone, a compound formed on irradiation of food containing fat." *Radiation Physics and Chemistry*, 52:39-42, 1998.

[17] Delincée, H. et al. "Genotoxicity of 2-dodecylcyclobutanone." Food Irradiation: Fifth German Conference, Federal Research Center for Nutrition, Karlsruhe, Germany, Nov. 11-12, 1998. BFE-R-99-01, pp. 262-269, 1999.

[18] Delincée, H. et al. "Genotoxicity of 2-alkylcyclobutanones, markers for an irradiation treatment in fat-containing food – Part I: cyto- and genotoxic potential of 2-tetradecylcyclobutanone." *Radiation Physics and Chemistry*, 63:431-435, 2002.

[19] Personal communication with Peter Jenkins, Center for Food Safety, Washington, D.C. Cited in written comments to the FDA submitted by the Center for Food Safety and Public Citizen, May 14, 2001.

[20] International Consultative Group on Food Irradiation, Op. cit.

[21] Statement from William Au, Department of Preventative Medicine, Medical Branch, University of Texas, to Scientific Committee on Food, European Commission, September 2002

[22] 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.

[23] Stevenson 1994, Op. cit.

[24] Stevenson, M.H. et al. "The use of 2-dodecylcyclobutanone for the identification of irradiated chicken meat and eggs." *Radiation Physics and Chemistry*, 42: 363-366, 1993.

[25] Delincée, H. "Rapid detection of irradiated frozen hamburgers." 12th International Meeting on Radiation Processing, Avignon, France, March 25-30, 2001.
[26] Gadgil, P. et al. "2-alkylcyclobutanones as irradiation dose indicators in irradiated ground beef patties." *Journal of Agricultural and Food Chemistry*. 50:5746-5750, 2002.
[27] Stevenson, M.H. "Identification of irradiated foods." *Food Technology*, 48: 141-144, 1994.
[28] Burnouf et al 2001, Op. cit.
[29] Tewfik, I.H. et al. "A rapid method (SFE-GC-MS) used to detect irradiated minced beef." 12th International Meeting on Radiation Processing, Avignon, France, March 25-30, 2001.
[30] Crone 1992, Op. cit.
[31] Stevenson, M.H. and Crone, A.V.J. "Irradiation detection." *Nature*, 344: 202-203, 1990.
[32] Stevenson, M.H. "Progress in the identification of irradiated foods." *Trends in Food Science & Technology*, 3: 257-262, 1992.
[33] Stevenson et al 1993, Op. cit.
[34] Lembke, P. et al. "Characterization of irradiated food by SFE and GC-MSD." *Journal of Agricultural and Food Chemistry*, 43: 38-45, 1995.
[35] Stewart, E.M. et al. "2-alkylcylcyclobutanones 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.
[36] Chow, C.K. (Ed.) *Fatty Acids in Foods and Their Health Implications*. New York: Marcel Dekker, 2000.