Center for Food Safety

660 Pennsylvania Ave., SE, Suite 302
Washington, DC 20003
www.centerforfoodsafety.org

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Division of Dockets Management (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Room 1061
Rockville, MD 20852

RE: Docket No. FDA-1999-F-2405 (formerly 1999F-5522)
Irradiation in the Production, Processing and Handling of Food

The Center for Food Safety appreciates the opportunity to file objections to the final rule designated by the above docket number. Our objections pertain to two major areas: 1) Adverse nutritional impacts of the final rule that FDA has failed to adequately analyze; and 2) Potential safety issues relating to radiation-insensitive pathogens that FDA has failed to analyze. For the sake of brevity, citations to references 1 to 61 cited in the FDA's final rule are denoted "Ref. #" and are not included in the References section at the end of this document. Other references not cited in FDA's rule are included in the References section. We first list specific objections. The analysis supporting these objections is provided below.

Objections:

1. FDA has failed to determine the magnitude of nutrient losses to be expected from irradiation of fresh spinach and iceberg lettuce at or near the maximum permitted dose of 4 kGy, undermining its analysis.
2. FDA has substantially underestimated the nutritional contributions of fresh spinach and iceberg lettuce to American diets, and therefore underestimated the impacts of irradiation-induced nutritional losses in these vegetables on American diets.
3. FDA has failed to conduct a cumulative assessment of irradiation-induced nutrient losses in fresh spinach and iceberg lettuce in combination with irradiation-induced nutrient losses in other foods already approved for irradiation, and should undertake such an assessment. Given the large number of items FDA is presently considering for irradiation approval in food additive petition 9M4697, cumulative assessments of this sort should be undertaken prospectively for the items covered in this FAP.
4. FDA has failed to determine whether irradiation of fresh spinach and iceberg lettuce as permitted in the final rule will increase the risk of food-borne disease from radiation-insensitive pathogens such as *Clostridium botulinum*, despite raising this potential safety issue in the discussion section of the final rule.
5. FDA has failed to consider alternatives to irradiation of fresh spinach and iceberg lettuce that would increase food safety without degrading the nutrient quality of American diets; in particular, FDA has failed to take action on a citizens' petition proposing such alternatives submitted in 2006.

Public Hearing:

Center for Food Safety requests a public hearing to address all of these objections.

CFS is particularly concerned with inadequacies in FDA's treatment of the adverse nutritional impacts of the rule on American diets. These inadequacies fall into three major categories:

1) FDA's analysis of irradiation-induced nutrient losses in fresh spinach and iceberg lettuce;
2) FDA's assessment of the nutritional impacts of these nutrient losses on American diets; and
3) FDA's failure to consider cumulative impacts of irradiation-induced nutrient losses in fresh spinach/iceberg lettuce in combination with nutrient losses in other foods already approved for irradiation, or pending approval. The discussion below focuses mainly on spinach.

Analysis Supporting Objection No. 1:

FDA fails to determine the magnitude of nutrient losses to be expected from irradiation of fresh spinach or iceberg lettuce at or near the upper limit approved in the rule: 4 kGy. According to FDA's Dr. Alison Edwards, speaking generally of the literature on the nutritional status of irradiated plant foods: "...the majority of experimental studies have focused on doses up to 2 kGy" (Ref. 33, p.4). The discussion in both Dr. Edward's memorandum and the FDA's rule cite studies mainly in the < 2 kGy level on vegetables and fruits, with occasional reference to studies at doses up to 3 kGy. Moreover, the great majority of studies cited refer to vegetables and fruits other than fresh spinach/iceberg lettuce.

Carotenoids/Vitamin A:

FDA's discussion of irradiation's impact on carotenoid levels in the final rule cites four studies: carrots irradiated at 2 kGy, mangoes and papayas at doses up to 2 kGy, broccoli at 2 and 3 kGy, and fresh spinach at up to 1 kGy. Dr. Edwards considers several others: pineapple at 2 kGy and carrots irradiated at 0.8 kGy (Ref. 33, p. 8). Of these six studies, then, only one involved irradiated spinach, and it was conducted at no higher than ¼ the approved dose of 4 kGy (Ref. 39), and is thus of limited value. Three of these six studies found reduced carotenoid levels even at these sub-4 kGy levels (carrots at 0.8 kGy, broccoli and mangoes/papayas). There is no discussion of the apparent discrepancy between no carotenoid loss in carrots at 2 kGy and "low to moderate losses in beta and alpha-carotene" (8% and 28% losses, respectively, after 5 days of storage) in carrots irradiated at less than half that dose, 0.8 kGy. In addition, discussion is limited mainly to "total carotenoid levels" with little breakdown data for various carotenoids with particular nutritional relevance, such as lutein/zeaxanthin. According to a USDA nutritional database, raw spinach contains 122 mcg lutein + zeaxanthin per gram spinach (USDA NDL-1). Low dietary intake and plasma levels of lutein and zeaxanthin have been associated with low macular pigment density and increased risk of age-related macular degeneration, and on this basis these carotenoids have been considered good candidates for designation as a "conditionally essential" nutrients (Semba & Dagenelie 2003).

FDA has considered only one study of irradiation's effect on carotenoid/Vitamin A levels in fresh spinach, and this single study was conducted at no higher than 1 kGy. FDA has thus failed to determine the magnitude of carotenoid/Vitamin A loss to be expected from irradiation of fresh spinach or iceberg lettuce at or near the 4 kGy maximum dose approved in the present rule.

Folate
FDA considers only two studies of folate loss in irradiated plant foods (Refs. 43 & 44). The only one involving fresh spinach found total folate losses of roughly 12% vs. the non-irradiated control at just 2.5 kGy (Ref. 44, Figure 1a)[1], just over half the approved dose. This study also found total folate losses of approximately 21% in fresh spinach irradiated at 5 kGy, which latter result FDA for some reason failed to report (Ref. 44, Figure 1a). These researchers also found a roughly 13% reduction in total folate levels in dehydrated spinach irradiated at 10 kGy relative to the non-irradiated control, which result FDA also failed to report (Ref. 44, Figure 1b). A second study involving irradiation of dehydrated spinach at 10 kGy (Ref. 43) found no loss of folate activity, a result that FDA did report. The results for dehydrated spinach are of little relevance given the greater sensitivity of fresh vs. dehydrated spinach to radiolytic damage (including nutrient loss) due to its much higher water content. Based on the single relevant study cited by FDA, folate losses in irradiated fresh spinach at the maximum approved irradiation dose of 4 kGy may be estimated at 17-18% (interpolating between 12% loss at 2.5 kGy and 21% loss at 5 kGy). A single study is not sufficient, however; additional research is needed to confirm or disconfirm this result.

Though iceberg lettuce contains considerably less folate than spinach (USDA NDL-2), lettuces as a group supply a larger percentage of folate than the spinach/greens groups to the average American diet (Ref. 33, p. 6). FDA does not consider any study of irradiation-induced folate loss in iceberg lettuce.

Vitamin K:
The third and final vitamin assessed by FDA in the rule is Vitamin K. FDA cites two 1961 studies. Ref. 41 identifies Vitamin K as one of the least radiation sensitive of the fat-soluble vitamins. This study involved irradiation of pure Vitamin K and other vitamins in isooctane solution rather than a food matrix, and so is of limited value for assessing irradiation-induced loss of Vitamin K in irradiated spinach or iceberg lettuce. Ref. 42 involved indirect measurement of Vitamin K activity in spinach and several other vegetables after freezing, irradiation at 28 or 56 kGy, or heat-processing. Vitamin K content was not measured directly, but rather estimated through measurement of the prothrombin times of chick plasma from chicks fed the various foods (after calibration of the chick model with reference doses of Vitamin K). The authors report anomalous results with this crude method, including: 1) increase in Vitamin K activity in irradiated spinach over time (activity value after 15 months of storage higher than directly after irradiation at both irradiation doses); and 2) values obtained in different assays frequently did not agree, as evidenced by extremely large variations (Ref. 42, Table 1). The authors themselves attribute these large variations to "difficulty in carrying out the Vitamin K assay procedure," indicating lack of confidence in their results.

FDA fails to consider conflicting results from the same period. For instance, Richardson et al (1956) (cited in Ref. 42) reported that the Vitamin K activity of diets containing small quantities of Vitamin K was markedly decreased by irradiation with sterilizing doses of gamma rays. Metta et al (1959) (also cited in Ref. 42) report Vitamin K deficiency in rats induced by the feeding of irradiated beef. A 2007 study raises similar questions (Hirayama et al 2007).

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[1] The results were not reported numerically, but rather only in bar graph form. The percentage values cited in the text were read off from the bar graphs in Figure 1a.

Irradiation is sometimes used to sterilize feed in experiments with germ-free laboratory animals. In this study, a standard mouse feed (AIN-76) formulated to contain 50 mcg/g Vitamin K_3 was pelletized and gamma-irradiated (50 kGy) and then fed to germfree mice. Half the mice died after 14 days on this diet, exhibiting symptoms typical of Vitamin K deficiency. Those animals fed irradiated AIN-76 supplemented orally with Vitamin K_3 starting on day 3 survived and did not display these symptoms. ***Analysis of the AIN-76 diet after pelletization and irradiation revealed no detectable level of Vitamin K_3.*** Germ-free mice fed another standard diet (AIN-93M) originally formulated to contain 750 mcg/g Vitamin K_1 experienced just 10% mortality after 15 days. However, Vitamin K_1 levels measured after pelletization and irradiation of the AIN-93 feed were 240 mcg/g, 68% less than the initial level. These results suggest the possibility that gamma irradiation at 50 kGy was responsible for completely eliminating the Vitamin K_3 content of the AIN-76 diet and substantially reducing the Vitamin K_1 content of the AIN-93M diet. This study strongly suggests the need for further research to determine the differential sensitivities of these two forms of Vitamin K to irradiation, particularly given the paucity of evidence cited by FDA, its age, and its poor quality. Studies involving direct detection of Vitamin K levels in spinach and iceberg lettuce irradiated at a range of doses relevant to the rule (4 kGy) is obviously needed.

Vitamin C

In the rule, FDA provides no assessment of Vitamin C loss from irradiation. Ref. 33 (Appendix B) reviews studies of irradiation-induced loss of Vitamin C in a range of fruits and vegetables, finding widely divergent results. One source of variation is attributed to whether ascorbic acid (AA) alone is measured, or AA plus dehydroascorbic acid (DHAA) to arrive at total ascorbic acid (TAA). In general, most studies find that irradiation causes substantial losses of AA but more modest declines in TAA. Also, differences between irradiated samples and non-irradiated controls in AA and/or TAA tend to diminish with increasing storage time.

A second source of variation is differential Vitamin C loss in different fruits and vegetables. For instance, in Ref. 33 Dr. Edwards cites a study by Graham and Stevenson (1997) which found a 6% loss in TAA in strawberries irradiated at 2 kGy after either 5 or 10 days of storage, though she fails to report results at 3 kGy. On the other hand, Dr. Edwards reports that a study by Fan et al (2003) on cilantro leaves irradiated at 1, 2 or 3 kGy found reductions in TAA of 45-50% vs. non-irradiated controls 14 days post-irradiation in samples “irradiated at the higher dose levels” more relevant to the present rule.

Given these widely divergent results for different vegetables, it is clearly necessary to examine data for the vegetables at issue in this rule. Fresh spinach contains 10-fold more TAA than iceberg lettuce (USDA NDL-1, USDA NDL-2), and is considered an “excellent source” of the vitamin. Unfortunately, in Ref. 33 Dr. Edwards reviews only one study of irradiation-induced Vitamin C loss in fresh spinach (Ref. 39). This study examined AA loss in fresh spinach irradiated at 0.3, 0.6 and 1.0 kGy. At 1 kGy, fresh spinach exhibited a roughly 20-fold lower AA level (0.22 mg AA per g) than the non-irradiated control (0.01 mg AA/g) on the day of treatment, and a 7-fold lower AA level (roughly 0.122 vs. 0.018 mg/g) after seven days of storage at 4 degrees C. While useful, this study is of limited utility for two reasons: 1) DHAA to arrive at TAA was not measured; and 2) The highest dose tested was only ¼ the maximum level approved in the present rule. However, it does suggest that irradiation would induce a substantial loss in

Vitamin C in fresh spinach at or near the maximum approved dose of 4 kGy that would continue for at least 7 days of storage. The only other relevant study that we have found is one reviewed by Diehl in Ref. 31 (p. 267, Figure 5), in which frozen spinach was irradiated at 0.5 and 5.0 kGy, with AA, DHAA and TAA measured on the day of treatment and after 1 month's storage. At 5.0 kGy, TAA was reduced by 32% vs. the non-irradiated control, a difference which narrowed considerably after one month's storage. One would of course expect a much larger drop in TAA in ***fresh*** spinach irradiated at this level, which is just over the maximum dose approved in this rule. Though data more relevant to the final rule would be desirable, the finding of a 20-fold and 7-fold decrease in AA levels in spinach (day of treatment, after 7 days storage, respectively) irradiated at just 1 kGy suggests that irradiation at or near the approved maximum dose would virtually eliminate AA content. The finding of a 32% drop in TAA in frozen spinach irradiated at just over the approved maximum suggests that ***total*** ascorbic acid levels would also be substantially lowered in more radiation-sensitive ***fresh*** spinach irradiated at or near the maximum approved level.

It is difficult to understand why FDA would approve irradiation at up to 4 kGy in the near complete absence of data on the nutritional impacts of this or higher doses on fresh spinach and iceberg lettuce. In the related context of radiolysis products, FDA set the rule that: "one can extrapolate from data obtained at high radiation doses to draw conclusions regarding the effects at lower doses," implicitly (and correctly) ruling out extrapolation in the opposite direction – just what it did here in its inadequate treatment of irradiation-induced nutritional losses. Several explanations – none adequate – seem possible.

First, FDA officers seem to take it as an article of faith that food processors will not irradiate fresh spinach or iceberg lettuce at doses above 2.0 kGy, even though they petitioned and received approval for irradiation at up to twice that level. Here is one typical example:

> "A realistic evaluation of the effects of irradiation on the nutritional quality of foods must, however, be based on the range of conditions ***likely to be encountered in actual practice.*** In evaluating the impact of irradiation on vitamin levels in spinach and iceberg lettuce, we have noted that the majority of experimental studies have focused on doses up to 2 kGy, which have been shown to achieve intended effects of reduction in microbial load without producing untoward effects on the texture or other organoleptic qualities of leafy green vegetables." (emphasis added, Dr. Edwards, Ref. 33, pp. 4-5).

We disagree with this approach. FDA must not base its analysis on speculation regarding "the range of conditions likely to be encountered in actual practice," that is, on the irradiation levels food processors are likely to employ today given the current constraints of food irradiation technology with respect to undesirable organoleptic changes. Rather, FDA must base its analysis on what the law as established in this final rule ***permits*** them to do. As we have shown in detail above for four nutrients (and as conceded by Dr. Edwards), the data on nutritional losses from irradiation at the upper end of the range approved by FDA are extremely weak and/or simply not available. We note also that the petitioners could easily have sought approval for a lower maximum dose, but chose not to do so. The fact that this expedited petition involved only two leafy vegetables with very similar limitations in this regard (i.e. undesirable organoleptic changes at roughly the same moderate doses) makes it still more likely that they had some good rationale for choosing this maximum dose rather than one (e.g. 1.5 or 2 kGy) at least somewhat better supported by the data. The original petition (FAP 9M4697) requested approval to irradiate a huge range of non-frozen and non-dry products, including a full range of raw and pre-processed fruits, vegetables and other plant products, at a maximum 4.5 kGy. 4.5 kGy may well be practically feasible from an organoleptic standpoint for some of the many food items in the original petition. However, when the petitioners "carved out" fresh spinach and iceberg lettuce from the original petition for expedited review, the rationale for requesting a dose higher than that which is practically feasible for these two vegetables in particular disappeared. If the petitioners, as FDA repeatedly assures us, will not apply radiation at or near 4 kGy to fresh spinach and iceberg lettuce, then they should not have requested approval at this maximum level, and FDA should not have granted it, in the virtual absence of solid experimental data on the nutritional impacts of such doses. Whatever the current constraints of food irradiation technology in this regard, FDA must be well aware that there is considerable research being undertaken into refinements of irradiation techniques designed to reduce undesirable organoleptic changes at any given dose (e.g. modified atmosphere packaging). Such refinements might push the "practically feasible" dose beyond what it is today, but might well do so at the cost of greater nutritional losses.

Another possible explanation for the failure to demand adequate data is that FDA simply doesn't take irradiation-induced nutritional losses seriously. Repeatedly, FDA officers downplay the nutritional effects of irradiation as less substantial than those caused by other (e.g. thermal) processing techniques, as if such observations were of any relevance to the rule. While superficially plausible, a moment's thought reveals the utter irrelevance of this line of thinking. Such comparisons would make sense only if irradiation were being proposed ***as a substitute for*** those other processing techniques. In that case, the putatively lesser impact of irradiation vs. methods it is intended to replace could be logically presented as an argument in its favor. But that is simply not the case here. Irradiation is proposed as an ***additional*** processing technique; irradiation-induced nutrient losses will be superadded to those from other industrial or home food processing methods. If irradiation causes a 20% drop in Nutrient X, for example, then irradiated fresh spinach will provide 20% less Nutrient X than non-irradiated fresh spinach, whether the irradiated fresh spinach is then eaten fresh or cooked at home. The fact that cooking may reduce Nutrient X by a larger amount than irradiation, say 50%, has absolutely no bearing on the question before the FDA. However, it should be noted that there are literature reports of "a more than additive (synergistic) effect of irradiation and heating," for example the finding of "higher heat-induced losses of tocopherol in irradiated samples … compared to the nonirradiated controls" for a number of foods (reviewed in Ref. 31, p. 256). FDA nowhere addresses such synergistic effects of irradiation and heating in the rule, a significant failing that should be redressed. Lacking any scientific relevance, FDA's repeated references to nutrient losses from thermal processing can only be interpreted as an attempt to put irradiation's nutrient-degrading effects in a context more familiar to consumers' own experiences (i.e. home cooking), and hence make irradiation appear more palatable.

Analysis Supporting Objection No. 2:

FDA's failure to determine the magnitude of nutrient losses to be expected from irradiation of fresh spinach and iceberg lettuce at the upper end of the approved dose range (i.e. roughly 2 to 4 kGy) undermines its analysis of the dietary impacts of irradiation-induced nutrient losses. This in itself justifies revocation of the rule while adequate studies are conducted and assessed. But there are other problems as well: no consideration of spinach's dramatically rising nutritional contribution to the average American diet over time; no consideration of subpopulations which rely more heavily on spinach than the statistically average American; and no consideration of cumulative nutritional degradation wrought by irradiation of fresh spinach and lettuce in combination with other foods already approved for irradiation (or, prospectively, pending approval for the same).

FDA employed two criteria to consider which nutrients deserved assessment: 1) Nutrients for which spinach/iceberg lettuce is an "excellent source" (defined as providing 20% or more of the Reference Daily Intake (RDI) or Daily Reference Value (DRV) per reference amount customarily consumed (RACC)); and 2) Nutrients for which spinach/iceberg lettuce contribute greater than 1 to 2 percent of the statistically average American's diet.

Table 1 shows the contribution of the RACC of fresh spinach (85 g) to the RDI of adults. Criteria #1 limits FDA to consideration of only 4 vitamins: A, C, K and folate. We note that FDA does not provide a rationale for considering only nutrients for which spinach is an "excellent source." A more conservative analysis would also consider those for which spinach is a good source, defined as nutrients for which the RACC contributes from 10 to 20% of the RDI/DRV. This would expand the list to include three additional vitamins: Vitamin E, riboflavin and Vitamin B_6.

Table 1

			RDI		Percent RDI	
Nutrient	**Amount 100 g**[1]	**Amount RACC (85 g)**	**Adult (highest)**	**Adult (lowest)**	**Adult (highest)**	**Adult (lowest)**
Vitamin A (mcg)	469	398.65	900	700	44%	57%
Thiamin (mg)	0.078	0.0663	1.2	1.1	6%	6%
Riboflavin (mg)	0.189	0.16065	1.3	1.1	12%	15%
Niacin (mg)	0.724	0.6154	16	14	4%	4%
Pantothenic acid (mg)	0.065	0.05525	5	5	1%	1%
Vitamin B6 (mg)	0.195	0.16575	1.7	1.3	10%	13%
Folate (mcg)	194	164.9	400	400	41%	41%
Vitamin C (mg)	28.1	23.885	90	75	27%	32%
Vitamin E (mg)	2.03	1.7255	15	15	12%	12%
Vitamin K (mcg)	482.9	410.465	120	90	342%	456%

[1] See USDA NDL-1 and USDA NDL-2.

For the second criteria, FDA relies primarily (except for Vitamin K) on a snapshot of dietary sources of various nutrients in the statistically average American's diet from over a dozen years ago (1994-1996) (Ref. 40). According to this snapshot, the contribution of the spinach/greens group to the nutrient composition of the average American diet was roughly 12% for Vitamin K (Ref. 33, pp. 9-10), 5.1% for Vitamin A (IU basis); 2.4% for folate; between 1 and 2% for Vitamin C; and less than 1% for Vitamin E, Vitamin B_6 and riboflavin. Lettuces as a group contributed 4.4% to total folate intake, 5th highest among all food groups, while iceberg lettuce contributed roughly 7% of the dietary intake of Vitamin K (Ref. 33, pp. 9-10). Interestingly, spinach ranks first in Vitamin K contribution to the diet (10.5% for men, 13.1% for women), while iceberg lettuce ranks 2nd for men (8.6%) and 4th for women (6.9%). Together, these two supply 19.1% and 20.0% of the dietary intake of Vitamin K for men and women, respectively.

It is interesting to note that the contribution of spinach/greens to intake of several of these vitamins has increased substantially over just the 5 years from 1989-1991, when an analogous survey was done, to 1994-1996. The corresponding spinach/green values for the 1989-1991 period, and the percentage increase from 1989-91 to 1994-96, are as follows: 2.8% for Vitamin A (up 82%, from position number 9 to number 3 among leading food groups for Vitamin A), 2.0 for folate (up 20%), and less than 1% for Vitamin C (up roughly 80-100%).[2] The 1989-1991 data do not include a value for Vitamin K, while the contribution of spinach/greens to the statistically average intake of Vitamins E, B_6 and riboflavin were not reported in 1994-1996, but apparently remained below 1%. Lettuces as a group contributed just 3.6% of folate in 1989-91, which rose 18% to 4.4% in 1994-96. Together, the spinach/greens and lettuces groups' contribution to folate has increased substantially from 5.6% in 1989-1991 to 6.8% - a substantial increase of 21% in just five years.

The rapidly increasing contributions of the spinach/greens group to intake of Vitamin A, folate and Vitamin C are borne out by surging consumption of fresh spinach over the past four decades, as documented by USDA (see Lucier et al 2004 for discussion below). USDA figures show that per capita use of total spinach (fresh and processed) increased by 34% from the 1970s (1.78 lbs per year) to the early years of this decade (2.39 lbs./year from 2000 to 2002). Particularly striking, however, is the increase in per capita consumption of ***fresh*** spinach, which nearly quintupled from the 1970s (0.32 lbs.) to the 2000s (1.46 lbs.). This reflects a huge shift in spinach consumption from predominantly processed forms in the 1970s (82% of all spinach) to predominantly fresh spinach in the 2000s (fresh spinach comprised 61% of all US spinach consumption from 2000 to 2002).

USDA reports an 18% increase in total spinach consumption from 1990 to 1995 (1.41 to 1.66 lbs. per capita, respectively). Since spinach dominates the spinach/greens group, this increase may well be responsible for a major part of the rising contribution of spinach/greens to the statistically average American's dietary intake of Vitamin A, folate and Vitamin C documented in the snapshots discussed above. It is interesting to note as well that total spinach consumption increased dramatically, by 44%, from 1995 to the average for 2000-2002 (from 1.66 to 2.39 lbs. per capita). This rise in total spinach consumption is comprised of a remarkable 118% increase in fresh spinach use (0.67 to 1.46 lbs per capita per year) and a slight decline in processed spinach use (0.99 to 0.94 lbs. per capita per year). An unknown but substantial portion of this increase in fresh spinach use is attributable to the rise of packaged salad mixes (from roughly $750 million in sales in 1995 to over $2 billion by 2002), of which (baby) spinach is a major component. Hence, much of the more than two-fold increase in fresh spinach consumption from 1995 to 2000-2002 is spinach that is consumed in the more nutritious fresh form that is the subject of this rule.

These figures and observations may be used to provide a rough approximation of the dietary contribution of spinach in the early years of this decade. As noted above, total spinach

[2] While the spinach/greens group did not make the list of food groups contributing at least 1% of Vitamin C in 1989-1991, it ranked 5th highest among 12 food groups contributing between 1 and 2% of Vitamin C in 1994-96.

consumption increased more than twice as much from 1995 to 2000-2002 (44%) as it did from 1990 to 1995 (18%). Moreover, there has been a dramatic increase in the proportion of spinach eaten in fresh vs. processed form over the more recent period.[3] One may therefore hazard an estimate that nutrient contributions from spinach from 1995 to 2000-2002 have increased by roughly 3-fold more than they did over the 1989-1991 to 1994-1996 period, based on: 1) a more than 2-fold greater increase in total spinach consumption over the later (44%) vs. the earlier (18%) period; and 2) a much higher proportion consumed in more nutritious fresh form from 1995 to 2000-2002. This would suggest, for example, that spinach contributed 6.9% more Vitamin A in the 2000-2002 period than it did in 1994-1996 (2.8% in 1989-1991 to 5.1% in 1994-1996: 2.3 percentage point rise multiplied by 3), or 12.0%. Similar calculations for the other nutrients yield contributions from spinach of roughly 3.6% for folate and from 4-5% for Vitamin C. In 2000-2002, spinach likely contributed over 1% or even 2% of the other vitamins for which spinach is a good source (Vitamin E, riboflavin and Vitamin B_6).[4]

To our knowledge, there is no evidence that the upward trend in spinach consumption discussed above has reversed or slowed. Hence, per capita spinach consumption is likely still higher today than in 2000-2002. The dramatic rise in consumption of one of our most nutritious vegetables is surely a heartening success story in an age marked by rampant consumption of junk foods and soft drinks and epidemic childhood obesity. Even so, we must put this success into perspective. USDA notes that in 2002, Americans consumed just 0.18 servings of dark green leafy vegetables per capita per day, far below (just 30%) the recommended level of 0.6 (Guthrie, JF 2004). In other words, it is the official policy of US government nutrition professionals that Americans should be eating more than three times the amount of dark leafy green vegetables than they do today (or did in 2002). The demonstrated and growing popularity of spinach makes it a natural candidate in efforts to close at least part of this gap. In the future, then, spinach may well make substantially greater nutritional contributions to American diets than it does today. It should also be noted that spinach is uniquely positioned to increase Americans' intake of precisely those vitamins we are most likely to lack. Of the six "shortfall" nutrients identified by USDA as those most likely to be lacking in the diets of Americans,[5] spinach is an "excellent source" of three (folate, Vitamins A and C) and a "good source" of one other (Vitamin E). School-age children are most likely to lack two of these nutrients (folate and Vitamin A),[6] and so could also benefit from more spinach.

Unfortunately, FDA makes no attempt to consider the recent trends outlined above concerning spinach's dramatically rising nutritional contributions to the American diet. Neither is there any prospective analysis that charts future trends in spinach consumption, perhaps as part of

[3] USDA figures show a modest decline in fresh spinach consumption from 1990 to 1995, which is more than made up for by an increase in processed spinach use over this period.
[4] A crucial assumption here is that the estimated increases in spinach's nutrient contributions are not diluted by increased consumption of other foods rich in the relevant nutrients. However, consumption of such other foods may just as easily have decreased as increased, in which case the estimates would be underestimates. While the method used to arrive at these estimates is admittedly quite crude, this should not distract attention from the basic point: a truly dramatic increase in (fresh) spinach consumption since 1995 means that it provided substantially more nutrients to the American diet in the period from 2000 to 2002 than it did in the 1994-96 period used as the basis for FDA's estimates of its nutrient contributions.
[5] See Table D1-2 at http://www.health.gov/dietaryguidelines/dga2005/report/HTML/D1_Tables.htm.
[6] See Table D1-4 at http://www.health.gov/dietaryguidelines/dga2005/report/HTML/D1_Tables.htm.

government efforts to enhance the nutritional adequacy of the American diet. Instead, FDA bases its assessment of irradiation-induced nutritional losses in fresh spinach on a 13-year old "snapshot" that misses the growing importance of this vegetable to the nutritional adequacy of American diets. FDA also fails to consider the impacts of irradiation on the nutritional status of subpopulations that rely more heavily on spinach for nutrition than the statistically average American, to which we now turn.

FDA's assessment of the impact of irradiation-induced nutrient losses in spinach is limited to a consideration of those impacts on the statistically average American. While this is a good starting point, it should be supplemented by consideration of subpopulations for which spinach contributes a greater-than-average amount of nutrients. We note that it is common practice to consider subpopulations in nutritional matters, and are surprised that FDA did not undertake such an analysis.

According to USDA (see Lucier et al 2004 for the following discussion), the average American consumed 2.37 lbs. of total spinach (fresh equivalent) in 2002, of which 1.49 lbs. (63%) was fresh spinach. However, this average obscures marked deviations in subpopulations. Below, we consider three such subpopulations: Asians, women 60 years of age or older, and vegetarians.

Asians comprise 3% of the U.S. population, and are the most rapidly growing ethnic group in the nation, their population having risen 50% from 1990 to 2000. Asians consumed on average 6.19 lbs. of total spinach per capita in 2002, 3.87 lbs. of it (63%) fresh spinach. On average, Asians consume over 2.6-fold times as much fresh spinach as the average American (3.87 vs. 1.49 lbs. per capita). Thus, it is reasonable to assume that spinach supplies a larger share of the nutrients in the average Asian's diet as well. We estimated above that in 2000-2002, spinach supplied roughly 12.0% of Vitamin A, 3.6% of folate, 4-5% of Vitamin C, and 1-2% of Vitamin E, riboflavin and Vitamin B_6 to the average American diet. Asians may well consume more non-spinach, dark leafy greens (with a nutritional profile similar to that of spinach) than the average American, so it is difficult to carry this rough analysis any further. However, it is probably not unreasonable to assume that the average Asian obtains twice as much Vitamin A (>20%), folate (7%) and Vitamin C (8-10%) from spinach as the average American.

The situation is similar for women 60 years of age and older, who represent nearly 9% of the U.S. population. They consumed on average 3.51 lbs. of spinach per capita in 2002, 2.67 lbs. of it fresh spinach (76%). This represents nearly 79% more than the average fresh spinach consumption (1.49 lbs). In general, both men and women over 40, comprising 39% of the U.S. population, consume substantially more spinach (both fresh and processed) than younger Americans.

A dietary survey carried out in the mid to late 1990s that involved nationally representative sampling of over 13,000 Americans found that 2.5% identified themselves as vegetarians (Haddad & Tanzman 2003). Of these, 0.9% reported no consumption of meat on the survey days. Dietary questionnaires revealed that vegetarians of the latter group consumed 2.3-fold more dark green vegetables than non-vegetarians. There was no breakdown for spinach or other dark green vegetables. On this basis, the increased contribution of spinach to nutrient intake of vegetarians would presumably fall in the same higher range as suggested above for Asians and women aged 60+.

To sum up, FDA has greatly underestimated the nutrient contributions of spinach to American diets through neglecting the dramatic rise in spinach consumption over the past 12 years, and through neglect of subpopulations with higher than-than-average spinach consumption. This, coupled with suggestive evidence of considerable irradiation-induced losses of nutrients like Vitamin A, folate and Vitamin C from irradiation of spinach at or near the maximum level approved by FDA, undermines its analysis of the rule's impact on the nutritional adequacy of American diets.

Analysis Supporting Objection No. 3:

The petitioner in this case, the Food Irradiation Council (FIC), had originally submitted a much broader petition (FAP 9M4697) requesting FDA to approve irradiation of an enormous array of pre-processed and so-called "ready-to-eat" foods. According to the FIC's lead member, the National Food Processors Association (NFPA), the original petition applied to foods that comprise as much as 37% of the food Americans consume each year.[7] The present rule is the result of a request to FDA to "carve out" for expedited consideration an extremely small subset of the foods covered by the original petition – fresh spinach and iceberg lettuce.

The adverse nutritional impacts on American diets resulting from irradiation of these two vegetables – while for some subpopulations significant – are obviously far less pronounced than impacts that would result from irradiation of the full range of foods covered by the original petition. The Center for Food Safety is concerned that if the petitioner repeats this process in the future – that is, breaks out small subsets of the foods covered in the original petition for separate, "expedited" consideration by FDA – it is likely that in each separate case, the adverse nutritional impacts from irradiation will appear to be relatively minor, even if those impacts are substantial and significant when considered cumulatively. Therefore, FDA obviously needs some mechanism to consider the cumulative impacts of nutritional losses from irradiation on the full range of foods approved for irradiation to date in its assessment of any particular irradiation approval petition. The alternative, piecemeal approach represented by this rule involving spinach and lettuce (assuming it becomes the norm) would likely result in a series of irradiation approvals – each for a small number of foods – that cumulatively could constitute substantial degradation of the nutritional quality of American diets. In conducting cumulative assessments of this sort, FDA should conservatively assume that the entire supply of the given food for which irradiation has been permitted will in fact be irradiated at the maximum permitted dose. The FDA's Dr. Alison Edwards employed this "100% commercial application" approach in her analysis of irradiation of spinach and iceberg lettuce (Ref. 33), though her analysis is undermined by lack of experimental data for nutritional losses at or near the maximum permitted dose of 4 kGy.

Analysis Supporting Objection No. 4

In the text of the final rule, FDA says it gave careful consideration to the question of whether "irradiation of iceberg lettuce and spinach …. could result in significantly altered microbial

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[7] See "Coalition petitions FDA to allow use of irradiation on ready-to-eat foods," Food & Drink Weekly, August 30, 1999. http://findarticles.com/p/articles/mi_m0EUY/is_34_5/ai_55621922

growth patterns such that these foods would present a greater microbiological hazard than comparable food that had not been irradiated." In fact, FDA considered only one such case: "whether the proposed irradiation conditions would increase the probability of significantly increased growth of, and subsequent toxin production by, *Clostridium botulinum* because this organism is relatively resistant to radiation as compared to non-spore-forming bacteria."

FDA's conclusion was that: "the possibility of increased microbiological risk from *C. botulinum* is extremely remote because: (1) The conditions of refrigerated storage necessary to maintain the quality of iceberg lettuce or spinach are not amenable to the outgrowth and production of toxin by *C. botulinum* and, (2) sufficient numbers of spoilage organisms will survive such that spoilage will occur before outgrowth and toxin production by *C. botulinum* (Refs. 48 and 60)."

The safety concern raised by FDA is that suppression of radiation-sensitive bacteria by irradiation might offer enhanced growth conditions (i.e. a competition-poor environment) for pathogens that are more resistant to irradiation, such as *C. botulinum*. FDA's analysis, however, fails to adequately address this concern.

First of all, FDA provides no discussion of radiation-insensitive pathogens other than *C. botulinum*, even though it provides no rationale for focusing exclusively on this single organism. This leaves it unclear whether: 1) *C. botulinum* is the only radiation-insensitive pathogen known to FDA; 2) FDA knows of other such organisms but had reasons for not considering them; or 3) FDA arbitrarily chose to consider only *C. botulinum*.

Second, the single study cited by FDA (Ref. 60) to support its conclusion that irradiation will not increase the risk of botulism in irradiated spinach/iceberg lettuce did not involve use of irradiation, and did not involve spinach or iceberg lettuce. In this study, the authors inoculated fresh-cut Romaine lettuce and shredded cabbage in vented and unvented packages with *C. botulinum* spores and stored them for 28 days under three temperature regimes: 4.4, 12.7 and 21 degrees C. The authors reported that nonvented packages of shredded cabbage became toxic after 7 days at 21 degrees C., while nonvented packages of Romaine lettuce became toxic after 14 days at 21 degrees C. No toxin was detected in samples stored at 4.4 or 12.7 degrees C. The authors concluded that at 21 degrees C, spoilage rendering the samples inedible occurred before toxin production. This study in no way addresses the safety concern raised by FDA: that elimination of spoilage and other bacteria via irradiation might provide enhanced growing conditions (i.e. a competition-poor environment) for radiation-insensitive pathogens such as *C. botulinum*, and thereby pose "increased microbiological risk" vs. non-irradiated spinach/lettuce. We reiterate also that the study did not involve either iceberg lettuce or spinach, but rather two different vegetables: Romaine lettuce and shredded cabbage. The fact that *C. botulinum* multiplied more rapidly on shredded cabbage (toxic after 7 days) than Romaine lettuce (toxic after 14 days) shows that this bacterium can have markedly different growth patterns on different vegetables of the same general type, underscoring the illegitimacy of extrapolating from data gathered on one vegetable to another.

The second reference provided by FDA (Ref. 48) is a memorandum by FDA's Dr. Robert Merker which discusses the study described above. Dr. Merker concedes that this study did not involve irradiation, but maintains that:

> "Irradiation would not change this conclusion, because spoilage organisms become easily established and ***attain previous levels within days of treatment***. … Eliminating some resident microorganisms, including pathogens, through irradiation would not lead to *C. botulinum* proliferation and toxin production, ***but rather to outgrowth of remaining spoilage organisms, which usually are reestablished shortly after treatment***" (emphasis added)

Dr. Merker's statement that spoilage microorganisms "attain previous levels within days of treatment" is contradicted by data he himself cites earlier in the same memo. Both FDA and Dr. Merker cite Zhang et al (2006) (Ref. 56) in another context. This study documents substantially lower total bacterial counts on irradiated vs. non-irradiated control lettuce that persist throughout the nine-day storage period. Zhang et al document total bacterial counts on lettuce irradiated at just 1.5 kGy that are over 3 orders of magnitude lower than levels on non-irradiated control lettuce on the day of treatment, over 4 orders of magnitude lower after 3 days of storage, over two orders of magnitude lower after 6 days of storage, and nearly 3 orders of magnitude lower after 9 days of storage (Ref. 56, Table 1 and associated discussion).

Dr. Merker acknowledges this finding earlier in his memo, as follows: "The authors followed numbers of viable bacteria for 9 days of storage, noting that ***for all groups, relative reductions persisted*** while total numbers of bacteria increased about 2-$\log_{10}$ throughout the period…" (emphasis added, Ref. 48, pp. 5-6).

The persistence of dramatically lower bacterial counts, including those of spoilage microorganisms, in irradiated produce is well-documented and indeed, is the basis for the frequently noted extended shelf-life of irradiated produce. Thus, Dr. Merker's statement that spoilage microorganisms "attain previous levels within days of treatment" is incorrect. According to data he himself cites, their levels remain two to four orders of magnitude (100 to 10,000-fold) lower than on non-irradiated controls through 9 days of storage. Thus, the question of whether the growth of *C. botulinum* or other radiation-insensitive pathogens present on irradiated fresh spinach or iceberg lettuce would be enhanced by this considerable suppression of competing bacteria, such as spoilage microorganisms, through irradiation remains unanswered. It should be noted that Zhang et al (2006) tested irradiated lettuce at a maximum does of 1.5 kGy, far below the maximum of 4 kGy permitted in the rule; thus, the disparity in bacterial load of irradiated vs. non-irradiated produce would very likely be still greater at higher doses.

Only properly conducted experimental studies involving irradiation of fresh spinach and iceberg lettuce inoculated with *C. botulinum* at a full range of doses up to and including 4 kGy, can address the safety concern raised, but not answered, by FDA in the rule.

Analysis Supporting Objection No. 5

The final rule at issue here is FDA's response to a request dated December 4, 2007 from the petitioners to give expedited consideration to the part of the original request dealing with irradiation of fresh spinach and iceberg lettuce. The final rule in effect trades off degradation of the nutritional quality of fresh spinach and iceberg lettuce for a putative enhancement in food safety, which is also true of food irradiation as whole. Unfortunately, FDA has chosen not to consider alternatives that would enhance the safety of a broad range of produce without degrading their nutritional quality. One example is a citizen petition filed with the FDA by Center for Science in the Public Interest on November 15, 2006,[8] over a year before the petitioners' expedited request. The petition calls on FDA to issue regulations to tackle the problem of microbial contamination of fresh fruits and vegetables at the source. Measures that FDA is urged to take include prohibition of applying raw manure to produce fields during the growing season; requiring that manure applied to food crops be composted first to destroy pathogens; and numerous other common-sense recommendations to prevent contamination of produce with pathogens. The FDA is urged to act on this petition as soon as possible.

[8] Posted at http://www.cspinet.org/new/pdf/fda_produce_petition.pdf.

References

Note: References cited and listed in full in FDA's final rule are not included here, but rather are referenced in the text of the comments as "Ref. #."

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