

# Packet 1

Recent studies conducted by the Federal Research Center for Nutrition in Karlsruhe, Germany, and co-funded by the International Consultative Group on Food Irradiation (ICGFI), have raised serious questions about the safety and wholesomeness of irradiated food.

A 1998 *in vitro* study found that a unique irradiation byproduct of palmitic acid was “clearly” cytotoxic and “clearly” genotoxic to human cells and to rat cells. The chemical, a cyclobutanone called 2-dodecylcyclobutanone (2-DCB), has not been found naturally in any food anywhere on Earth.<sup>1</sup> For reasons that have yet to be adequately explained, the World Health Organization (WHO) misstated and dismissed the findings of this study in its recent report on high-dose irradiated food.<sup>2</sup>

A 1998 *in vivo* study found that 2-DCB caused “significant DNA damage” to rats that consumed the chemical. Researchers stated that these results “urge caution, and should provide impetus for further studies.”<sup>3</sup> These studies are currently underway. For reasons that have yet to be explained, neither ICGFI nor the WHO has publicly commented on the findings of this study.

These findings take on a greater significance in light of the fact that numerous studies conducted since 1990 have identified 2-DCB in food irradiated at doses as low as 0.5 kGy,<sup>4</sup> including beef, pork, lamb, chicken, eggs, mangoes, papayas, peanuts, instant soup powder, and freshwater, saltwater and anadromous fish.<sup>5, 6, 7, 8</sup> This chemical 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 for up to 13 years.<sup>9</sup>

The findings take on an even greater significance in light of the fact that palmitic acid is a naturally occurring ingredient in virtually all types of meat (including fish and shellfish), vegetables, fruit, grains, dairy products and vegetable oils.<sup>10</sup> In relation to petitions currently pending before the FDA, palmitic acid occurs in:

- dozens of ready-to-eat foods, including sauces, pizzas, baked goods and snack foods.<sup>11</sup>
- crustacean shellfish in appreciable quantities, representing 16 percent of the fatty acids in Alaskan shrimp, 14 percent in queen crab, and 9.2 percent in king crab.<sup>12</sup>
- molluscan shellfish in appreciable quantities, representing the highest percentage of fatty acids in American oysters (28.9 percent), ocean quahaug (23.6 percent) and European oysters (22.4 percent); and the third-highest percentage of fatty acids in Pacific scallops (19.3 percent).<sup>13</sup>
- various types of poultry in varying quantities: 0.28-3.82 g/100g in chicken, 0.23-1.61 g/100g in turkey, 1.22-9.58 g/100g in duck, and 1.47-6.95 g/100g in goose. In each of these types of poultry, palmitic acid is the fatty acid with the second-highest concentration: 21.6-24.6 percent in chicken, 21.2-21.6 percent in turkey, 26.0-28.3 percent in duck, and 22.6-28.3 percent in goose.<sup>14</sup>
- various types of meat in varying quantities: 1.42 g/100g in beef, 1.49 g/100 g in pork, 1.01 g/100 g in lamb, and 0.49 g/100 g in veal. In each of these meats, palmitic acid is the fatty acid with the second-highest concentration: 25.96 percent in beef, 24.39 percent in pork, 23.56 percent in veal, and 22.82 percent in lamb.<sup>15</sup>

In March, the Federal Research Center for Nutrition released an abstract of an *in vitro* study on human cells that revealed cytotoxic effects of two additional types of cyclobutanones — 2-tetradecylcyclobutanone (2-TCB) and 2-tetradecenylcyclobutanone (2-TDCB) — which are unique irradiation byproducts of stearic acid and oleic acid, respectively. *In vivo* experiments with rats are being planned.<sup>16</sup>

Based on these findings — and because of the German researchers’ warning — the FDA should refrain from considering all pending petitions until the ongoing and planned experiments are completed. The agency must act with caution until more is known about the potential cytotoxicity and genotoxicity of these chemicals.

## Notes

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<sup>1</sup> Delincee, 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.

<sup>2</sup> *High-dose irradiation*. Report of a Joint FAO/IAEA/WHO Study Group. Geneva: WHO, 1999.

<sup>3</sup> Delincee, H. et al. "Genotoxicity of 2-dodecylcyclobutanone." Food Irradiation: Fifth German Conference, Karlsruhe, November 11-13, 1998.

<sup>4</sup> Stevenson, M.H. Identification of irradiated foods. *Food Technology*, 48: 141-144, 1994.

<sup>5</sup> Ibid.

<sup>6</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>7</sup> Tewfik, I.H. et al. A rapid supercritical fluid extraction for the qualitative detection of 2-alkylcyclobutanones in gamma-irradiated fresh and sea water fish. *International Journal of Food Science and Nutrition*, 50: 51-56, 1999.

<sup>8</sup> Lembke, P. et al. Characterization of irradiated food by SFE and GC-MSD. *Journal of Agricultural and Food Chemistry*, 43: 38-45, 1995.

<sup>9</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>10</sup> Chow, C.K. ed. *Fatty Acids in Foods and Their Health Implications*. New York: Marcel Dekker, 2000.

<sup>11</sup> Ibid.

<sup>12</sup> Ibid.

<sup>13</sup> Ibid.

<sup>14</sup> Ibid.

<sup>15</sup> Ibid.

<sup>16</sup> Delincee, H. et al. "Genotoxicity of 2-alkylcyclobutanones, markers for an irradiation treatment in fat-containing food." 12<sup>th</sup> International Meeting on Radiation Processing, March 25-30, 2001, Avignon, France.

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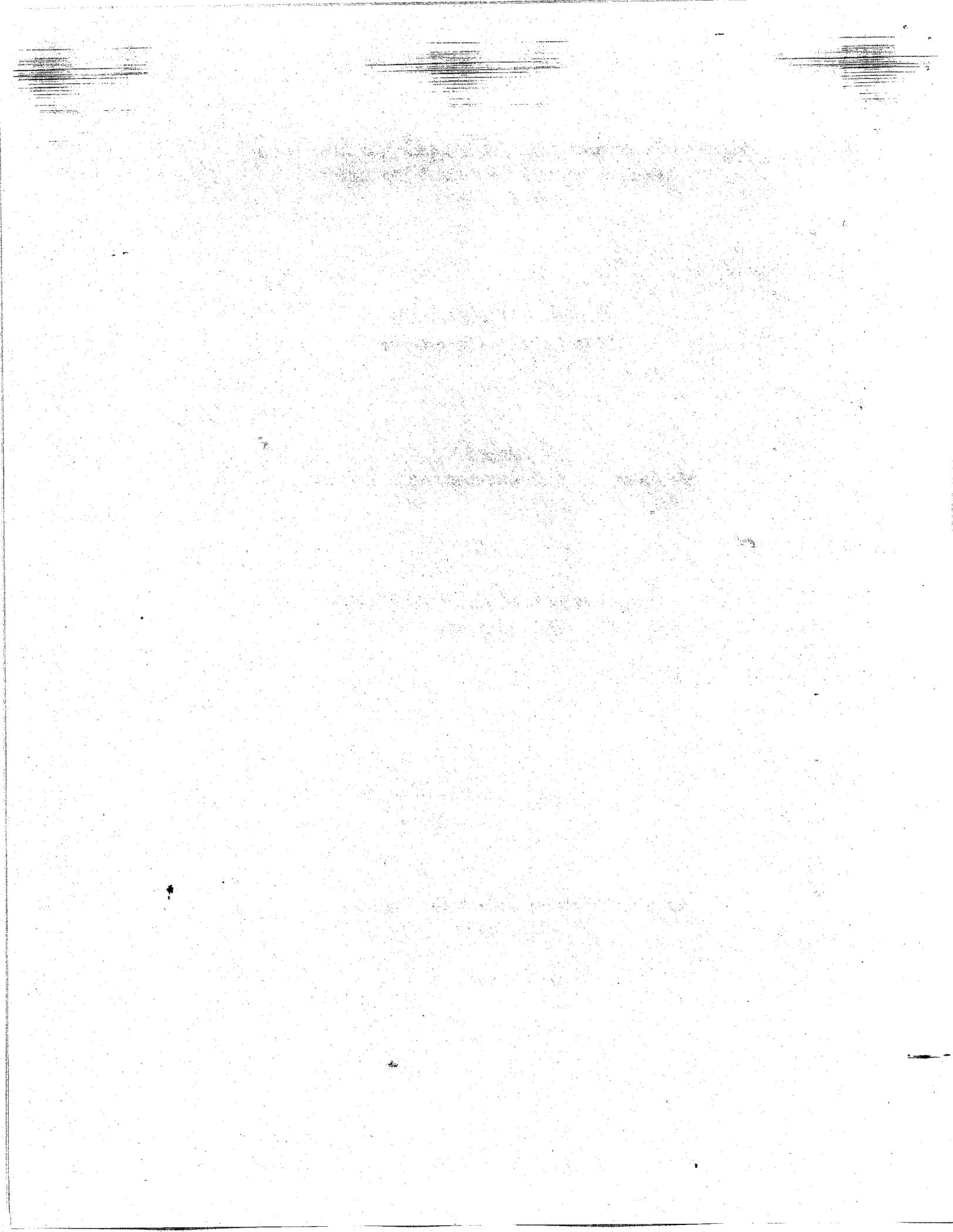
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# Genotoxicity of 2-dodecylcyclobutanone

Henry Delincée, Beatrice-Louise Pool-Zobel and Gerhard Rechkemmer

Institut für Ernährungsphysiologie der Bundesforschungsanstalt für Ernährung  
[Institute for Nutritional Physiology of the Federal Nutrition Research Institute]  
Haid-und-Neu-Str. 9, D-76131 Karlsruhe

## Summary

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* O.157: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 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-dodecylcyclobutanones 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-dodecylbutanone (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-dodecylcyclobutanone (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.

## Introduction

Of late there has been growing interest in the treatment of foods with ionizing radiation. The irradiation can help improve the hygienic quality of the food and prevent diseases that otherwise could be caused by consumption of foods contaminated with parasites or pathogenic microorganisms. Furthermore, the irradiation of certain foods facilitates an improvement in the storage life and reduces the spoilage rate [Diehl, 1995]. A growing number of countries have approved the use of ionizing radiation for numerous products [Anon., 1998]. Within the EU, one can expect harmonization of the legal regulations of the member states with regard to foods and food components treated with ionizing radiation. As a first step, irradiation of dried aromatic herbs and spices is to be permitted in all EU nations. This development is based in part on the positive evaluation of the procedure by the World Health Organization. In a 1992 position

statement, WHO stated that "foods that have been treated with ionizing radiation and produced according to good manufacturing practice (GMP) are to be regarded as safe in terms of health and satisfactory from the perspective of nutritional physiology." Numerous studies and animal feeding experiments, as well as experiments on volunteer test subjects, support this conclusion [WHO, 1994]. Taking account of the studies available to date, a new expert committee concluded in 1997 that "even irradiation of foods with high doses (> 10 kGy) may be judged safe and satisfactory in terms of nutrition" [WHO, 1997, 1998]. In recent years, there has also been increasing interest in analytical techniques to determine whether a product has been irradiated [Delincée, 1998]. For example, a research team in Northern Ireland has determined that certain lipolytic digestion products — namely, the 2-alkylcyclobutanones [LeTellier and Nawar, 1972] — might be products that are unique to irradiation and therefore hold great promise as markers of irradiation treatment [Stevenson et al., 1990, Stevenson, 1996]. As a result of irradiation, the acyl-oxygen bond in triglycerides is cleaved, with formation of 2-alkylcyclobutanones with the same number of carbon atoms as the initial fatty acid and with the alkyl group in ring position 2. For example, 2-dodecylcyclobutanone and 2-tetradecylcyclobutanone are formed from palmitic acid and stearic acid, respectively. Although 2-methylcyclobutanone has been identified following ultrasound treatment of *Hevea brasiliensis* latex, for example [Nishimura et al., 1977], cyclobutanones have not yet been detected in non-irradiated foods [Stevenson, 1996]. However, since cyclobutanones do occur in irradiated foods — for example, at levels of 0.3-0.6 µg 2-dodecylcyclobutanone/g fat/kGy in chickens [Stevenson et al., 1990, 1993; Boyd et al., 1991; Crone et al., 1992 a, b, 1993; Stevenson, 1996] — it is necessary to characterize their potentially toxic features and undertake a risk evaluation.

In this study, the so-called "comet assay," a new test procedure that detects DNA damage in individual cells by means of microgel electrophoresis, has been employed as the toxicological test procedure [McKelvey-Martin et al., 1993; Fairbairn et al., 1995]. Rat colon cells<sup>1</sup>, tissue in which tumors can be generated under certain nutritional conditions, were used as the target cells.

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<sup>1</sup> [Translator's note] The German "Dickdarm" used here can be translated "large intestine" or "colon" (the latter a segment of the former); "colon" has been translated since the authors used the unmistakable "Kolon" in the same context in the "Summary."

## Materials and Methods

### *Materials*

The test substance, 2-dodecylcyclobutanone (2-DCB) was synthesized according to the specifications of Boyd et al. (1991).

### *In vivo experiment*

Male Sprague-Dawley rats ( $\approx 250$  g) were obtained from Charles River Wiga GmbH (D-97633 Sulzfeld) and kept under the usual conditions. The rats were randomly divided into 4 groups. Two groups of six animals each received 2-DCB via pharyngeal probe: the first group received 1.12 mg/kg body weight (BW), the second group 14.9 mg/kg BW. A group of three animals served as negative control, and received the solvent of 2-DCB, namely 2% dimethyl sulfoxide (DMSO) in physiological sodium chloride solution (5 ml/kg BW). The fourth group with three animals was employed as positive control, and received 15 mg 1,2-dimethylhydrazine (DMH)/kg BW (dissolved in physiological sodium chloride solution, 5 ml/kg BW). The feeding and treatment regimen employed here has been described (Pool-Zobel et al., 1996). After 16 hours of exposure — which was determined to be the optimal period of time for the formation of DNA damage in colon cells caused by DMH and measurable by the comet assay [Pool-Zobel, 1996] — the colon was removed from the rats and the colon cells isolated by means of enzymatic digestion [Brendler-Schwaab et al., 1994].

### *Cytotoxicity*

The potential cytotoxicity of 2-DCB to the cells of the colon was checked with the aid of the trypan blue vitality test, a rapid and simple method to differentiate between living and non-living cells [Pool et al., 1990; Pool-Zobel et al., 1994].

### *Comet assay*

DNA damage to the colon cells was determined by means of single-cell microgel electrophoresis (comet assay) [Pool-Zobel et al., 1994; Pool-Zobel and Leucht, 1997]. For each data point, 50 cells per slide and 3 slides per determination were analyzed. The evaluation was carried out on a fluorescence microscope with the image processing system of Perceptive Instruments (Halstead, Great Britain). The DNA distribution in the comet was calculated as “% tail intensity” and “tail moment” — the latter a product of the proportion of DNA in the tail and the length of the comet tail [Fairbairn et al., 1995]. With more severe damage to the DNA, the proportion of DNA in the tail, and hence also the “% tail intensity” and “tail moment,” increase.

### *Determination of the quantity of substance administered*

Two different concentrations of 2-DCB were selected. The low concentration was meant to model radiation pasteurization (e.g. with 3 kGy), while the higher concentration was intended to represent radiation sterilization (60 kGy).

For the radiation pasteurization (3 kGy) of fresh chicken, we assumed formation of  $\approx 1.5$   $\mu\text{g}$  of 2-DCB/g fat. Since palmitic acid represents only about 1/5 of the fatty acids in chicken, the total quantity of cyclobutanones was roughly projected to be 5 times as great. If one assumes at the same time that all of the fat that a person consumes is irradiated (according to the DGE<sup>2</sup>-

<sup>2</sup> DGE = Deutsche Gesellschaft für Ernährung = German Nutrition Association

Nutrition Report 1996, a man weighing 70 kg consumes an average of 104 g fat/day, or 1.49 g fat/kg BW), this would lead to a 2-DCB content of  $1.5 \mu\text{g} \times 5 \times 1.49 = 11.2 \mu\text{g}$  of 2-DCB/kg BW.

With a safety factor [Classen et al., 1987] of 10 for individual differences, and an additional factor of 10 to account for differences between various species (here, rat/human), the expected no-effect level (NOEL) for radiation pasteurization lies at

$$11.2 \mu\text{g} \times 10 \times 10 = 1.12 \text{ mg 2-DCB/kg BW.}$$

Similarly, one would expect a NOEL of

$$20 \mu\text{g} \times 5 \times 1.49 \times 10 \times 10 = 1.49 \text{ mg 2-DCB/kg BW}$$

for the radiation sterilization (60 kGy) of frozen chicken. This calculation is based on formation of  $\approx 20 \mu\text{g}$  of 2-DCB/g fat for radiation sterilized (60 kGy), frozen ( $-46^\circ \text{C}$ ) chicken [Crone et al., 1992a].

## Results

The trypan blue vitality test did not reveal any cytotoxic effects on the colon cells from the 2-DCB that was administered. The vitality of the treated cells was on the same order of magnitude ( $\approx 90\%$ ) as the cells of the negative control group, which were treated with DMSO alone.

On the other hand, DNA damage from 2-DCB was observed in the comet assay. In the evaluation of the comets, both as "% tail intensity" and as "tail moment," the DNA damage exceeded that found in the negative control group. In the group of six animals that received the lower concentration of 1.12 mg 2-DCB/kg BW, two of the animals exhibited increased DNA damage, while four of the animals exhibited values like those of the control group (Fig. 1a).

When the results of the experimental group animals were combined, there was no significant difference relative to the negative control group (Fig. 1b). At the higher concentration of 14.9 mg 2-DCB/kg BW, an increased level of DNA damage was also detectable in the group, relative to the negative control group (Fig. 1b). While the increase in DNA damage is slight compared to the positive control group, which received DMN as alkylating agent, one must recall that the latter is a strong and specific rat colon carcinogen.

Fig. 1a Effect on Individual Animals

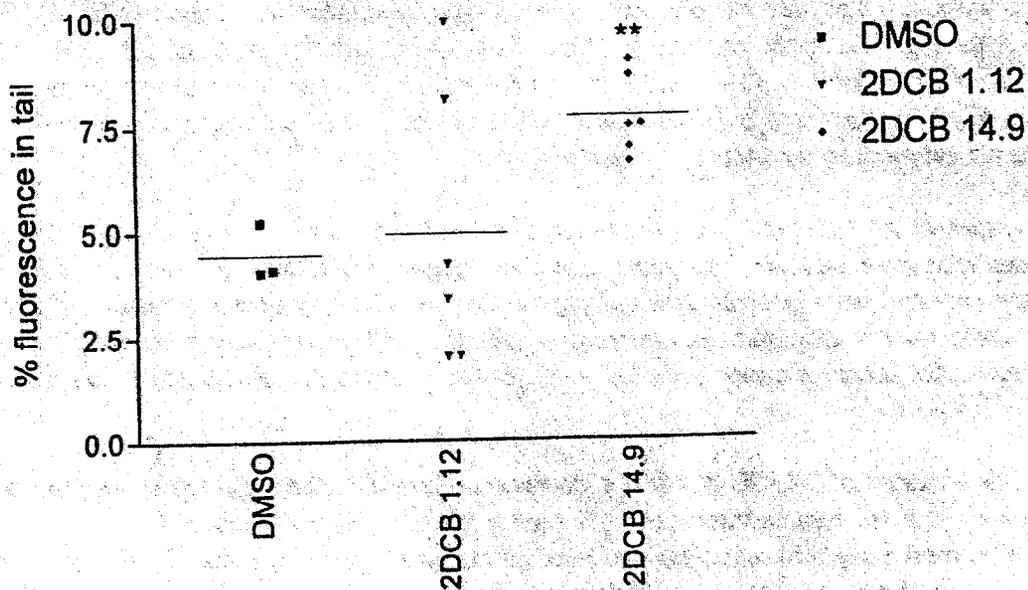
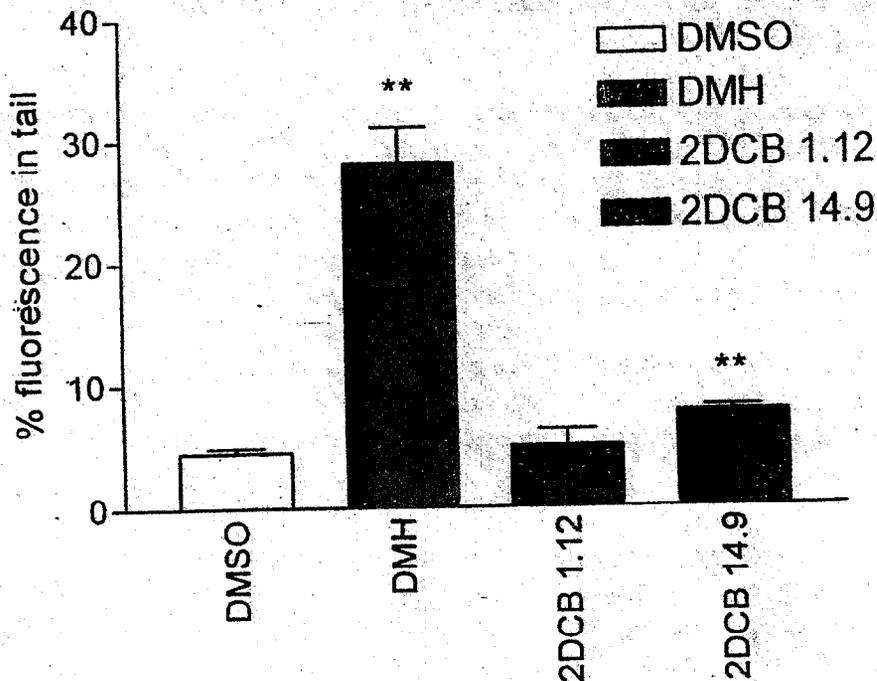


Fig. 1b Effect on Groups of Animals



Figures 1a, b DNA single-strand breaks in rat colon cells from the action of 2-dodecylcyclobutanone and DMSO, or DMH. Administered with pharyngeal probe 16 hours before isolation of the colon. (\*\*  $p < 0.01$  significantly different from the negative control with DMSO; unpaired, two-sided Student's t-test,  $n = 3-6$ ).

## **Discussion**

Initial *in vitro* experiments with 2-dodecylcyclobutanone, which at various concentrations was applied to rat colon cells as well as colon cells from human biopsies, have shown that 2-DCB leads to DNA damage [Delincée and Pool-Zobel, 1998]. Although the concentrations of 2-DCB that were used, ranging from 0.30 — 1.25 mg/ml, are large in comparison to the expected consumption of  $\mu\text{g}$  quantities of 2-DCB, further clarification is needed to determine whether these results are relevant to the safety of irradiated foods.

The *in vivo* experiments that were just conducted likewise show DNA damage to colon cells at higher concentrations of 2-DCB. Of course, one must keep in mind that not every instance of DNA damage proves to be a precursor to damage severe enough to generate a tumor, or leads to mutations in tumor-relevant genes. Furthermore, possible DNA repair processes and other cytotoxic events, for instance apoptosis, play a role before lesions become manifest and cell degeneration is initiated.

In addition, the quantity of 2-DCB that was administered here is to be regarded as very high. A projection shows that the concentration of 14.9 mg/kg BW in humans corresponds to consumption of more than 800 radiation-sterilized (60 kGy) broiler chickens. This comparison raises the question of whether the safety factors must in fact be  $10 \times 10$ . With several food ingredients (e.g. selenium), this concept would lead to deficiency symptoms, since the amount required in rats, for example, is about 25% of the toxic dose [Classen et al., 1987]. With lower safety factors, and hence lower test concentrations of 2-DCB, there would no longer be any detectable DNA damage.

It should be mentioned once again that in many animal feeding experiments with irradiated foods in which it is known that cyclobutanone was also in the feed, no evidence has been found to indicate an injury from irradiated foods that have been consumed. Typical in this regard is the Raltech study in the USA [Thayer et al., 1987], in which several generations of mice and dogs were fed with radiation-sterilized chicken. This study also included nutrition-physiological, teratological and genotoxic experiments on various species of animal.

In each case, it is necessary to check the relevance of the results that have been obtained. It is striking that the variation in observations is much greater at the low dose than the high dose, which in the latter case entails statistical significance. This must also be clarified.

## **Conclusion**

High concentrations of 2-dodecylcyclobutanone lead to DNA damage in colon cells that is detectable with the comet assay. The requisite concentrations are very much higher than those that can be reached through the consumption of irradiated foods that contain fat. The results urge caution, and should provide impetus for further studies.

## **Note of thanks**

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## GENOTOXIC PROPERTIES OF 2-DODECYLCYCLOBUTANONE, A COMPOUND FORMED ON IRRADIATION OF FOOD CONTAINING FAT

Henry DELINCÉE and Beatrice-Louise POOL-ZOBEL

Institute of Nutritional Physiology, Federal Research Centre for Nutrition,  
Engesserstr. 20, D-76131 Karlsruhe, Germany.

### ABSTRACT

When food containing fat is treated by ionizing radiation, a group of 2-alkylcyclobutanones is formed. These components contain the same number of carbon atoms as their precursor fatty acids and the alkyl group is located in ring position 2. Thus, from palmitic acid 2-dodecylcyclobutanone is derived. To date, there is no evidence that the cyclobutanones occur in unirradiated food. Therefore, these components cannot be considered inherent to food, and for questions pertaining to risk assessment of irradiated food it would be advisable to determine the genotoxic and toxic potentials of cyclobutanones. Measurements of DNA damage in cells exposed to 2-dodecylcyclobutanone, employing the single cell microgel electrophoresis technique, have been carried out. *In vitro* experiments using rat and human colon cells indicate that 2-dodecylcyclobutanone in the concentration range of about 0.30 - 1.25 mg/ml induces DNA strand breaks in the cells. Simultaneously, a concentration related cytotoxic effect is observed as was determined by trypan blue exclusion. To which extent these *in vitro* findings are of relevancy for the *in vivo* human exposure situation needs to be investigated in further studies. *In vivo* tests in rats a

### KEYWORDS

Food irradiation; cyclobutanone; genotoxicity; comet assay

### INTRODUCTION

Food irradiation is a thoroughly tested technique and numerous studies have led to the conclusion "that irradiated food produced in accordance with established good manufacturing practice can be considered safe because the process of irradiation will not lead to changes in the composition of the food that, from a toxicological point of view, would have an adverse effect to human health" [WHO, 1994]. It is well-known that some radiolytic products are formed in very low quantities, which may cause some health hazards only if consumed in amounts much higher than actually present in irradiated food. Nevertheless, it is desirable to gain knowledge on the toxic potential of the individual radiolytic products formed. Since the very great majority of radiolytic products also are found in native or otherwise processed food, many toxicological evaluations of these radiolytic products have been carried out in the past, and are set in perspective to other levels of human exposure.

Twenty-five years ago, it was reported that on irradiation of triglycerides, a cyclic compound is formed of the same carbon number as the esterified fatty acid. This compound was identified as the 2-alkylcyclobutanone [LeTellier and Nawar, 1972]. Recently, these compounds have also been identified in irradiated food, and they were proposed to be a marker of the irradiation treatment [Stevenson *et al.*, 1990; Stevenson, 1996]. In fact, an analytical detection method for irradiated food based on the formation of 2-alkylcyclobutanones in fat-containing food has now been standardized on a European level (EN 1785 : 1996). It is claimed that e.g. 2-dodecylcyclobutanone (2-DCB) derived from palmitic acid is radiation-specific and has never been detected in any non-irradiated or microbiologically spoiled food [Stevenson, 1996]. Maybe that improved analytical techniques in future will find 2-alkylcyclobutanones also in otherwise treated foodstuffs at extremely low levels. However, at present it is known that these compounds are especially produced in a dose-dependent manner in irradiated food, and therefore an assessment of the health hazard of these 2-alkylcyclobutanones would be advisable.

In this paper, the genotoxic potential of 2-DCB was assessed using the "comet assay", which measures DNA strandbreaks in cells [McKelvey *et al.*, 1993; Fairbairn *et al.*, 1995]. According to a parallelogram approach [Pool-Zobel *et al.*, 1994], the test compound 2-DCB will be subjected to *in vitro* studies using rat and human colon cells, and subsequently an *in vivo* study with rats will be carried out. We used primary rat and human colon cells since the colon is an important target tissue for many food-related cancers. In this paper, the first *in vitro* estimations of the genotoxic potential of 2-DCB are reported.

#### EXPERIMENTAL

2-DCB was obtained, synthesized as described by Boyd *et al.* (1991), from Dr. C.H. McMurray (The Department of Agriculture for Northern Ireland, Belfast, UK). Rat colon cells were freshly isolated from rat colon using an *in situ* / *ex vivo* digestion procedure [Brendler-Schwaab *et al.*, 1994]. Human colon cells were isolated from biopsies [Pool-Zobel and Leucht, 1997]. Rat and human colon cells were incubated with 2-DCB in the concentration range of 0.30-1.25 mg/ml for 30 minutes at 37°C. Both the cytotoxicity test using the method of trypan blue exclusion and the DNA comet assay were performed as described by Pool-Zobel *et al.* (1994) and Pool-Zobel and Leucht (1997).

#### RESULTS AND DISCUSSION

A novel technique to detect genotoxic effects of chemicals is the comet assay, a micro gel electrophoresis of single cells to measure DNA damage. Freshly isolated colon cells were chosen since they are metabolically competent and expected to convert chemicals as in *in vivo*-like conditions.

Cytotoxicity of 2-DCB in rat colon cells is observed at increasing concentrations as shown in Fig. 1a. Toxicity was apparent at 1.25 mg/ml as a reduction in the percentage of viable cells (absolute viability below 80 %). Relative viability (based on 100 % viable cells in the untreated control at 0 mg 2-DCB) gave similar results.

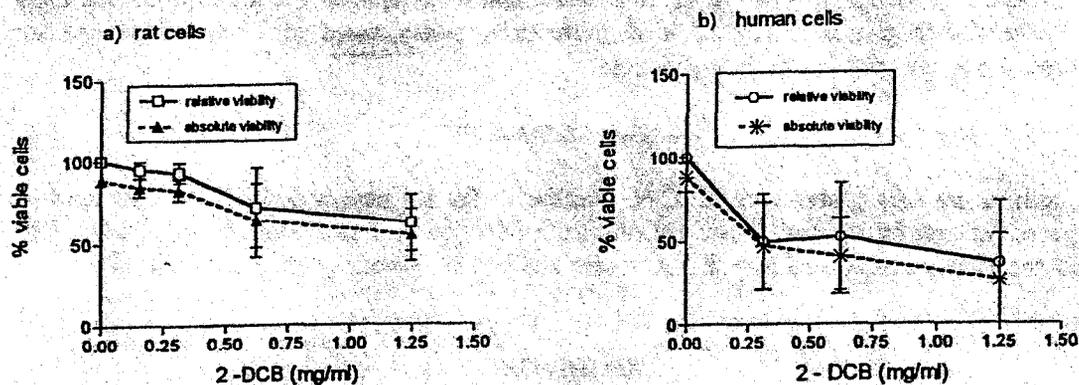


Fig. 1 a,b Viability of colon cells incubated with 2-DCB at various concentrations for 30 minutes at 37°C (means  $\pm$  SEM of 3 independent experiments).

In further experiments the DNA damage under the given experimental conditions - DNA single strand breaks - were measured in rat colon cells (Fig.2). The tail moment of the comets, which is a function of "tail length" and "intensity of fluorescence in the tail", was chosen as the parameter for DNA damage [Fairbairn *et al.*, 1995].

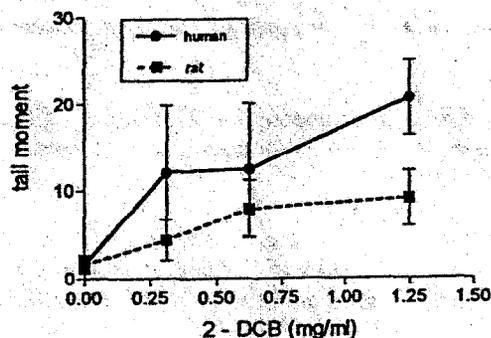


Fig. 2 DNA single strand breaks in rat and human colon cells induced by 2-DCB at various concentrations for 30 minutes at 37°C. (means  $\pm$  SEM of 4 rats and 3 separate human colons, 50 cells for each point)

Fig. 1b shows the viability of 2-DCB on human colon cells (from biopsies), and a cytotoxic effect with increasing dosage is clearly demonstrated. Human colon cells seem to be more sensitive than rat colon cells, since the viability is decreased to less than 50% at the highest concentration of 2-DCB (i.e. 1.25 mg/ml) tested. The higher sensitivity of human colon cells is also found in response to DNA damage, higher tail moments being measured (Fig.2).

These *in vitro* results clearly indicate a genotoxic effect of 2-DCB. However, concentrations tested are very high compared with actual human intake. Amounts of 2-DCB in irradiated food will vary dependent on radiation dose and other irradiation parameters, storage and storage conditions, and of course on the amount and kind of fat in the food. For chicken, amounts of about 0.3 - 0.6  $\mu\text{g}$  2-DCB / g lipid / kGy have been reported. In highly irradiated chicken meat from the Raltech study (mean radiation dose about 58 kGy), amounts of 17  $\mu\text{g}/\text{g}$  lipid were still

found after 12 years of storage [Crone *et al.*, 1992]. In the Raltech study no adverse effects attributable to the irradiation treatment were observed [Thayer *et al.*, 1987]. Thus, a possible risk from 2-DCB must be at a very low level. In order to assess and quantitate this minimal risk from the intake of 2-DCB with irradiated food, more experiments than these preliminary ones are required. An *in vivo* test in rats is in progress.

#### ACKNOWLEDGMENT

The authors are very grateful to Dr. C.H. McMurray for the supply of 2-DCB. This work is supported in part by the International Consultative Group on Food Irradiation (ICGFI). The skilful technical assistance of Mrs. R. Lambertz and Mr. M. Knoll is highly appreciated.

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# Identification of Irradiated Foods

Electron spin resonance spectroscopy and detection of 2-alkylcyclobutanones are two approaches available to identify foods that have been irradiated

M. Hilary Stevenson

UNTIL THE MID-1980s, LIMITED ATTENTION WAS directed toward using the changes that may be induced in food treated with ionizing radiation as a means of differentiating between irradiated and unirradiated products. Lack of emphasis in this area was at least partly due to the fact that detection methods were considered unnecessary because food would be irradiated in licensed facilities and documentation would accompany the irradiated food throughout the food chain (Anonymous, 1984). However, progress in commercialization of the process, greater international trade in irradiated food, differing regulations relating to use of the technology in many countries, and consumer demand for clear labeling of irradiated food highlighted the need for tests that could be applied to the food itself.

During the past few years, significant progress has been made in the development of detection methods. As well as the individual efforts of research teams in many countries, international cooperation in this field has been noteworthy. The European Community (EC), through its Community Bureau of Reference (BCR), has set up a collaborative program to develop methods to identify irradiated food. It has hosted a number of workshops (Raffi and Belliardo, 1991; Leonardi et al., 1992) and funded collaborative trials (Raffi, 1992; Sanderson et al., 1993). On a worldwide basis, the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture has a coordinated program on analytical detection methods in irradiation treatment of food (ADMIT) which has promoted cooperation in this area (IAEA, 1990, 1992).

## Criteria for a Detection Method

The criteria which an ideal detection method should meet have been clearly documented (IAEA, 1990). For example, the test should (1) be specific for irradiation and not influenced by other processes or storage, (2) be accurate and reproducible, (3) have a detection limit below the minimum dose likely to be applied to the food, (4) be applicable to a range of products, (5) be quick and easy to perform, and (6) be capable of providing an estimate of irradiation dose.

In practice, it is difficult to fulfill all these requirements, since the changes occurring in food subjected to irradiation are small and often similar to those induced by other processes such as cooking. There is no general method applicable to all foods; thus, a range of tests based on microbiological, biological, physical, and chemical changes in food are being developed to complement each other and reinforce the probability of detection (Delincée, 1991; Raffi and Belliardo, 1991; Leonardi et al., 1992; IAEA, 1992).

This article will discuss work carried out in our laboratory on (1) the use of electron spin resonance (ESR) spectroscopy for the detection of irradiated food containing bone or shells and (2) the formation of 2-alkylcyclobutanones in fat-containing foods. A summary of other procedures which have been subjected to collaborative testing is also included.

## ESR Spectroscopy

ESR spectroscopy is used to detect unpaired electrons in reactive species such as free radicals. Generally, the latter are so short-lived that they cannot be detected; but if they are trapped in hard, relatively dry components of a food, such as

bone, shell, or seeds, their presence can be confirmed by ESR spectroscopy.

**• Food Containing Bone.** When food containing bone is irradiated, free radicals are trapped in the crystal lattice of the bone, and they give an ESR signal with a characteristic shape independent of the origin of the bone (Dodd et al., 1988; Goodman et al., 1989; Raffi et al., 1989; Stevenson and Gray, 1989). The factors which might influence the formation and stability of the radiation-induced signal have been examined to demonstrate that the technique complies with the requirements of an ideal detection method.

The variables studied have included (1) age of chicken (Gray et al., 1990); (2) bone site within the carcass (Gray and Stevenson, 1990); (3) storage conditions (Stevenson and Gray, 1989); (4) cooking in a convection oven before and after irradiation (Gray and Stevenson, 1989a); (5) irradiation dose (Stevenson and Gray, 1989); (6) dose rate (Gray and Stevenson, 1991); and (7) temperature of irradiation (Stevenson and Gray, 1990).

The specificity of the radiation-induced signal has been confirmed, since no other system investigated has given a signal of similar shape. In addition, it has been detectable under all conditions examined and at doses well below those likely to be used commercially. The feasibility of using the ESR technique for estimating dose was demonstrated when it was shown that the intensity of the signal induced in chicken bone increased as irradiation dose increased up to 10 kGy (Stevenson and Gray, 1989). Nevertheless, because intensity of the radiation-induced signal may be affected to some extent by the factors outlined above, the ability to accurately estimate dose in samples with an unknown processing history will not be simple. Reirradiation of bone samples has been proposed as a means of eliminating the necessity of knowing the background of a sample, but extrapolation to original dose may still be affected by the storage history of the sample and the time between irradiation and detection (Dodd et al., 1988; Desrosiers et al., 1990).

Besides chicken bone, ESR has been used in primary food products. It has also been shown that ESR can be used to detect irradiation in irradiated products (Stevenson and Gray, 1989b). It has been shown that the method may also be used to detect irradiation in irradiated burgers.

**• Food Containing Shells.** The radiation-induced signal can also be detected in shells. However, the signal in shells is complex because of the presence of the  $\mu\text{m}$  signal, and careful manipulation of the software in the spectrometer is needed to isolate the signal due to irradiation. Although the intensity of the signal decreases with storage at 4°C, there is no difficulty in detecting it even 28 days after irradiation, by which time the product has spoiled and is no longer acceptable (Stewart et al., 1992). The signal is not generated by boiling samples in water for 3 min, and despite the fact that the intensity of samples cooked after irradiation is reduced, it is still possible to isolate the radiation-induced signal (Stewart et al., 1993).

The situation with food containing shells is more complex than for products containing bone because there is evidence that the nature of the free radicals formed and hence the shape of the ESR signal are species dependent (Fig. 1). This diversity of ESR signal shape will present problems of unambiguous identification of irradiation in samples whose origin is unknown.

—Continued on next page

The author is Principal Scientific Officer, Food and Agricultural Chemistry Research Div., Dept. of Agriculture for Northern Ireland, and Reader, The Queen's University of Belfast, Newforge Ln., Belfast BT9 5PX, Northern Ireland, United Kingdom.

### Detection of 2-Alkylcyclobutanones

LeTellier and Nawar (1972) reported the presence of 2-alkylcyclobutanones in triglycerides irradiated at high doses (60 kGy). These cyclic compounds have the same number of carbon atoms as the fatty acids from which they are formed, and the alkyl group is in ring position 2. This work provided the theoretical basis for the cyclobutanone method which was developed in our laboratory. Since most foods contain at least some fat, the method should be applicable to a wide range of foods. Preliminary studies have shown that the method has the potential to detect irradiated exotic fruits such as mangos, using the seeds as the source of fat, but the method may not be applicable to all fruits and vegetables.

Using chicken meat as a model for fat-containing food, a procedure has been developed for the isolation and detection of cyclobutanones in samples irradiated at doses well below 10 kGy (Stevenson et al., 1990; Boyd et al., 1991). The initial work concentrated on 2-dodecylcyclobutanone, which is formed from palmitic acid on irradiation. Because cyclobutanone standards are not available commercially, 2-dodecylcyclobutanone had to be synthesized.

The analytical method involved extraction of the lipid fraction using hexane, fractionation on a deactivated Florisil column, and identification using gas chromatography-mass spectrometry in the selected ion monitoring mode for ions  $m/z$  98 and 112.

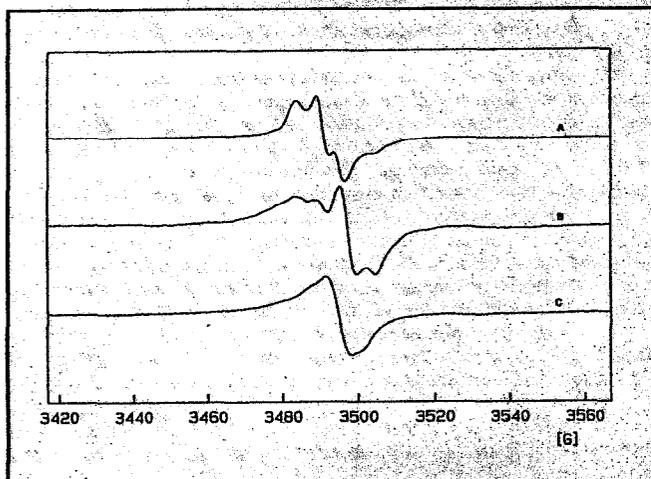


Fig. 1—Isolated Radiation-Induced Signal from products receiving a 5-kGy dose: (a) Mediterranean crevette, (b) pink shrimp, and (c) Norway lobster

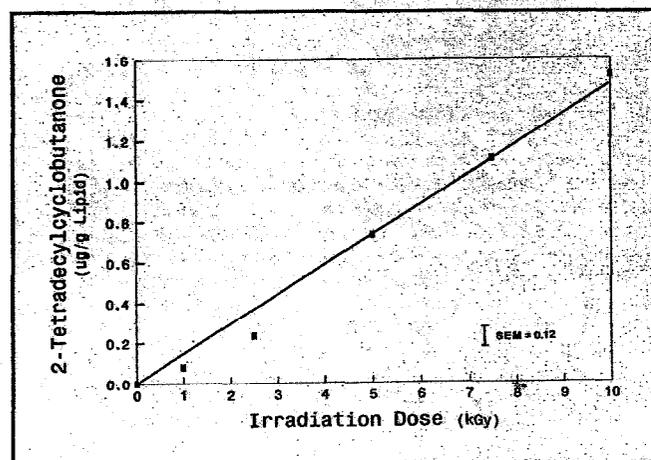


Fig. 2—Effect of Irradiation Dose on the amount of 2-tetradecylcyclobutanone formed in chicken meat. SEM = standard error of the mean

2-Dodecylcyclobutanone 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. Specificity of the compound as a marker for irradiation treatment was demonstrated when it was shown that it was not produced by cooking (Crone et al., 1992a); by packaging in air, vacuum, or carbon dioxide (Stevenson et al., 1993); or during storage (Crone et al., 1992a, b). Although the compound is partly destroyed by cooking, there is no difficulty in detecting it in cooked, irradiated samples (Boyd et al., 1991; Crone et al., 1992a). As irradiation dose increased up to 10 kGy, the amount of 2-dodecylcyclobutanone formed in fresh chicken increased linearly (Crone et al., 1992a; Stevenson, 1992). A similar response was found in samples irradiated at frozen temperatures, although the amounts present at each irradiation dose were slightly lower (Stevenson et al., 1993).

This work has now been supplemented and extended following synthesis of 2-tetradecylcyclobutanone, which is formed from stearic acid on irradiation. As with 2-dodecylcyclobutanone, the amount of 2-tetradecylcyclobutanone formed in chicken meat increased with increasing irradiation dose (Fig. 2), although the amounts present were lower and reflected the lower concentrations of stearic acid in chicken meat.

Preliminary experiments have shown that these two cyclobutanones are also present in pork, lamb, and beef (Table 1). Except for pork, the amounts of 2-dodecyl- and 2-tetradecylcyclobutanone formed were in a ratio similar to that of the precursor fatty acids (Table 2). The amount of 2-dodecylcyclobutanone present in pork was lower than expected relative to the amount of 2-tetradecylcyclobutanone produced, and the effect was further exaggerated when the pork was irradiated frozen.

Although the reason for these observations is unknown, the position of palmitic and stearic acid on the glycerol backbone may influence the quantity of cyclobutanone formed. In pork lipid, there is a tendency for palmitic acid to be preferentially bound at position 2 of the glycerol backbone, while stearic acid is most likely to be attached to position 1 (Gunstone et al., 1986). In this position, the stearic acid may be more easily cleaved and cyclized, hence producing more 2-tetradecylcyclobutanone. The even greater amounts of 2-tetradecyl- relative to 2-dodecylcyclobutanone in frozen irradiated pork may be due to closer packing of triglyceride molecules in the frozen state (Gunstone et al., 1986).

Interest in application of the cyclobutanone method to detect irradiated liquid whole egg was stimulated by the potential use of irradiation to control *Salmonella* in egg products. Both 2-dodecyl- and 2-tetradecylcyclobutanone were detected in liquid egg irradiated at 2.5 kGy (Fig. 3), and the amounts of the compounds were in a ratio similar to that of

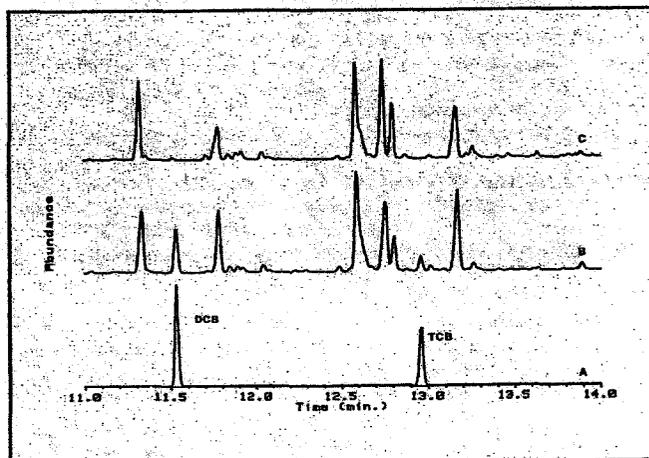
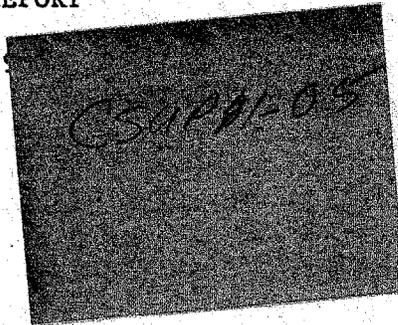


Fig. 3—Selected Ion Monitoring of the sum of ions  $m/z$  98 and 112 from (a) standards of 2-dodecylcyclobutanone (DCB) and 2-tetradecylcyclobutanone (TCB); (b) pasteurized, irradiated (2.5 kGy) liquid egg; and (c) pasteurized liquid egg

RECOMMENDATIONS FOR EVALUATING  
THE SAFETY OF IRRADIATED FOODS

FINAL REPORT

JULY 1965



Prepared for the Director, Bureau of Foods, FDA

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Division of Toxicology

## TESTING

Foods irradiated at doses above 100 Krad and comprising more than 0.01% of the diet are estimated to contain URPs in sufficient quantity to warrant toxicological evaluation. The non-mammalian mutagenicity tests offer a level of sensitivity not practically attainable in whole animal tests, and recalling that many URPs may be similar chemically to substances occurring naturally in foods, these tests are considered appropriate tools to evaluate the potential carcinogenicity of irradiated foods. The tests recommended are 1) gene mutations in bacteria, with and without metabolic activation, 2) gene mutations in cultured mammalian cells, 3) DNA repair in mammalian cells, and 4) recessive lethal mutations in *Drosophila*. These tests are considered to be the minimum battery. Requests for substitutions for any of the above tests should be justified and will be considered on a case by case basis.

Because of the anticipated low level of individual radiolytic products present in the whole irradiated food, the above tests must be performed on extracts in which the concentration of radiolytic products is maximized. Also, many of the radiolytic products from polysaccharides and proteins will be large molecules and will not penetrate the cell membrane in the in vitro systems, hence the use of enzyme digests is recommended prior to the concentration of URPs.

In addition to the short-term mutagenicity tests, foods irradiated at doses above 100 krad must be evaluated in 90-day feeding studies in two species (one rodent, one non-rodent). The 90-day rodent test should include in utero exposure. To assure that the test animals are exposed to the highest concentration of radiolytic products possible, the irradiated food may be lyophilized and incorporated into the animal diet at the highest concentration that does not compromise the nutritional requirements of the test species (see Appendix IV). It is not necessary to test enzyme digests of the irradiated food in these tests since each test animal provides digestion of food components before systemic absorption occurs. Higher doses of particular radiolytic products may be obtained if the selectively extracted and concentrated material used in the short-term tests is employed; however, it is recognized that much greater quantities would be needed for in vivo testing and thus would make this latter suggestion extremely difficult and expensive to effect in any practical sense.

## DEPARTMENT OF HEALTH AND HUMAN SERVICES

## FOOD AND DRUG ADMINISTRATION

## 21 CFR Part 179

[Docket Nos. 81N-0004 and 84F-0230]

## Irradiation in the Production, Processing, and Handling of Food

AGENCY: Food and Drug Administration.

ACTION: Final rule; denial of requests for hearing and response to objection.

**SUMMARY:** The Food and Drug Administration (FDA) is denying the requests that it has received for a hearing on the final rules that amended the food additive regulations to authorize the use of gamma radiation for the treatment of pork to control *Trichinella spiralis* and for the treatment of certain other foods. After reviewing the objections to the two final rules and the requests for a hearing, FDA has concluded that none of the objections has provided the information necessary to justify a hearing. FDA, however, is amending the language in the regulation that describes minor dry ingredients that may be radiation sterilized because objections and experience have shown that this language is ambiguous.

**DATES:** The amendment in 179.26(b) (21 CFR 179.26(b)) is effective December 30, 1988; written objections on the amendment and requests for a hearing on the amendment by January 30, 1989.

**ADDRESS:** Written objections on the amendment to the Dockets Management Branch (HFA-305), Food and Drug Administration, Rm. 4-62, 5600 Fishers Lane, Rockville, MD 20857.

**FOR FURTHER INFORMATION CONTACT:** Clyde A. Takeguchi, Center for Food Safety and Applied Nutrition (HFF-330), Food and Drug Administration, 200 C St. SW., Washington, DC 20204, 202-472-5740.

**SUPPLEMENTARY INFORMATION:****I. Background**

In the Federal Register of July 22, 1985 (50 FR 29658), in response to a petition by Radiation Technology, Inc., FDA issued a final rule authorizing the irradiation of fresh pork to control *Trichinella spiralis*. FDA based its decision on data in the petition and in its files. The agency had published a notice announcing the filing of the petition (FAP 4M3789) in the Federal Register of July 23, 1984 (49 FR 29682).

In the Federal Register of April 18, 1986 (51 FR 13376), FDA issued a final rule, referred to herein as the "omnibus

rule," that: (1) permitted manufacturers to use radiation at doses not to exceed 1 kilogray (kGy) (100 krad) to inhibit the growth and maturation of fresh foods and to disinfest food of arthropod pests; (2) permitted manufacturers to use radiation at doses not to exceed 30 kGy (3 Mrad) to disinfect dry or dehydrated aromatic vegetable substances (such as spices and herbs) of microorganisms; (3) required that foods that are irradiated be labeled to show this fact both at the wholesale and at the retail level; and (4) required that manufacturers maintain process records of irradiation for a specified period and make such records available for FDA inspection. FDA initiated this action by publishing a proposal in the Federal Register of February 14, 1984 (49 FR 5713).

**A. Requests for hearing on final rules**

Section 409(f) of the Federal Food, Drug, and Cosmetic Act (the act), 21 U.S.C. 348(f), provides that, within 30 days after publication of an order relating to a food additive regulation, any person adversely affected by such an order may file objections specifying with particularity the provisions of the order considered objectionable, stating reasonable grounds for the objection, and requesting a public hearing on such objections.

Under 21 CFR 171.110 of the food additive regulations, objections and requests for a hearing are governed by 21 CFR Part 12 of FDA's regulations. Under 21 CFR 12.22(a), (1) each objection must be submitted on or before the 30th day after the date of publication of the final rule; (2) each objection must be separately numbered; (3) each objection must specify with particularity the provision of the regulation or proposed order objected to; (4) each objection on which a hearing is requested must specifically so state; failure to request a hearing on an objection constitutes a waiver of the right to a hearing on that objection; and (5) each objection requesting a hearing must include a detailed description and analysis of the factual information to be presented in support of the objection. Failure to include a description and analysis for an objection constitutes a waiver of the right to a hearing on that objection.

FDA received 59 objections to the irradiated pork rule and 245 objections to the omnibus rule. Many of the objections expressed general opposition to food irradiation but identified no substantive question to which the agency can respond. Because these objections failed to raise any basis on which to question the validity of the final rules, the agency is denying them.

Seventeen objections to the irradiated pork rule and 53 objections to the omnibus rule pointed to a specific aspect of the rule but did not request a hearing. Twenty objections to the irradiated pork rule and 12 objections to the omnibus rule requested a hearing. These objections are addressed below.

Some of the objections requested a stay of the regulations. In the Federal Register of February 23, 1987 (52 FR 5450), FDA denied these requests because the public interest did not require a stay. FDA evaluated each of the contentions made in support of a stay and concluded that they failed to create significant doubts about the safety of the food irradiated under the conditions of either of the two regulations.

**B. Standard for granting a hearing**

The criteria for deciding whether to grant or deny a hearing are stated in 21 CFR 12.24(b). The regulation states that a hearing will be granted when the material submitted meets the following:

(1) The objection raises a substantial issue for hearing.

(2) The objection raises issues that have not been resolved by the rule.

(3) The objection provides adequate information to justify resolution of the factual issue in the way sought by the person.

A hearing will be denied if the Commissioner concludes that the data and information submitted are insufficient to justify the factual determination urged, even if accurate.

(4) Resolution of the factual issue in the way sought by the person is adequate to justify the action requested. A hearing will not be granted on factual issues that are not determinative with respect to the action requested, e.g., if the Commissioner concludes that the action would be the same even if the factual issue were resolved in the way sought, or if a request is made that a final regulation include a provision not reasonably encompassed by the proposal.

(5) The action requested is not inconsistent with any provision in the act or any regulation in this chapter particularizing statutory standards. The proper procedure in those circumstances is for the person requesting the hearing to petition for an amendment or waiver of the regulation involved.

no basis for the wholesale approval of irradiation. Since FDA's regulation did not require studies to test for the long-term health impact of these chemicals, it is in violation of the Food, Drug and Cosmetic Act, and evidence will be presented at the public hearing.

(HEI Para. L3.) (Emphasis by HEI.)

In this objection, HEI has made a number of allegations about the significance of FDA's decision not to require toxicological testing before concluding that irradiation of food in the circumstances set forth in § 179.26 is safe. However, HEI has failed to make an adequate proffer to support a hearing on any of these allegations.

Under FDA's regulations, a hearing will not be granted on the basis of mere allegations. (21 CFR 12.24(b)(2)). Consistent with this regulation, the relevant case law provides that where a party requesting a hearing only offers allegations without an adequate proffer to support them, the agency may properly disregard those allegations. *General Motors Corp. v. FERC*, 856 F.2d 791, 798 n. 20 (D.C. Cir. 1981). For example, FDA need not grant a hearing on HEI's claim that each irradiated food item has its own individual URP's because HEI has not presented any evidence to support this claim.

Furthermore, HEI's allegation that BFIFC "assumed" (emphasis by HEI) that " \* \* \* URP's would not constitute more than 3 parts per million concentration in food \* \* \* is also without support. As noted in the final rule, BFIFC based its estimate of the likely concentration of URP's in foods on a review of experimental data showing the amount and type of chemical change likely to be caused by a given amount of radiation (51 FR 13376 at 13377). HEI has not presented any evidence that challenges the basis for BFIFC's analysis or the agency's reliance on that analysis.

HEI asserts that the concentration of URP's is not the crucial factor but again has not provided any evidence or rationale to support its assertion. Even if HEI is correct that cancer can theoretically be initiated by a single URP or carcinogenic chemical, to justify a hearing, HEI would have to provide some evidence that would reasonably link low levels of URP's to the causation of cancer. HEI has not presented any such evidence.

As discussed earlier in this document and in the omnibus rule, FDA examined all available data from animal feeding studies with irradiated foods and found no link between irradiated food and cancer (51 FR 13376 at 13378). Therefore, HEI's assertion is a mere allegation that is not supported by any evidence. FDA

will not grant a hearing on the basis of such an assertion (21 CFR 12.24(b)(2)).

HEI's allegation that FDA has not presented scientific evidence that radiolytic products are chemically and toxicologically similar to known natural food components is untrue. The agency did cite specific articles on the radiation chemistry of food components in the proposed omnibus rule (49 FR 5714 at 5721, Ref. 7 to 12; see also 51 FR 13376 at 13380) and included other references in the administrative file (Docket No. 81N-0004). The agency considered this information in its safety evaluation of radiolytic products.

While FDA has the ultimate burden of proof when it approves the use of a food additive, in the sense that the agency must find the additive to have been shown safe, once the agency makes a finding in a listing document, the burden shifts to an objector to come forward with evidence that calls that finding into question. *American Cyanamid Co. v. FDA*, 806 F. 2d 1307, 1314-1315 (D.C. Cir. 1979). To justify a hearing, HEI would have to present some evidence that suggests that there are significant toxicological or chemical differences between radiolytic products and known natural food components. HEI has failed to present any such evidence and, thus, has not provided a basis for a hearing.

HEI contends that until carcinogenicity and mutagenicity studies are performed on concentrated radiolytic products, as suggested by Epstein and Gofman in a letter to the editor of *Science* (Ref. 17), there is no basis for the wholesale approval of irradiation. Epstein and Gofman stated that, "Stable radiolytic products could be extracted from irradiated food by various aqueous and nonaqueous solvents, which could then be concentrated and subsequently tested."

BFIFC, in its report (Ref. 5, p. 18), explicitly considered testing requirements, including the option of testing extracted and concentrated radiolytic products. Based on its review of the available literature dealing with the identity, amount and potential toxicity of radiolytic products, BFIFC recommended that such testing was not necessary to assure the safety of foods irradiated at doses below 1 kGy or of minor ingredients irradiated at doses below 50 kGy because of the low potential concentration of radiolytic products in such foods. The agency, in the omnibus rule, agreed with the recommendation and concluded that foods irradiated under the conditions of the regulation are safe, and that no additional toxicological testing should be required (51 FR 13376 at 13378).

HEI has not justified a hearing on this conclusion. Epstein and Gofman's letter merely presents a general assertion. HEI has not supported it with any evidence that the levels of radiolytic products formed in food irradiated under the conditions of the regulation would be so high as to require that toxicological testing be done or to call into question FDA's conclusion that foods that have been irradiated are safe. Therefore, HEI has not justified a hearing on this issue under 21 CFR 12.24(b)(2).

Finally, HEI asserts that because FDA did not require long-term animal studies, it is in violation of the act. This is a legal issue. Thus, it cannot serve as a basis for a hearing because a hearing will be granted only on the basis of a substantial issue of fact, not on issues of policy or law (21 CFR 12.24.(b)(1)).

FDA discussed applicable sections of the act in the omnibus rule. The agency stated that, "Section 409 of the act lists the [safety] criteria which must be considered by the agency before a food additive regulation is issued. The statute does not prescribe what safety tests should be performed but leaves that determination to the discretion of scientists." (51 FR 13376). As stated above, FDA's scientists have concluded that foods irradiated at the levels permitted need not be tested toxicologically, and the agency agreed with this conclusion (51 FR 13376 at 13378). HEI has not cited any authority that contradicts FDA's conclusions about the type of data that is necessary to support the approval of the use of a food additive. Therefore, FDA finds that this aspect of HEI's objection is without merit.

#### C. Animal Feeding Studies

As stated in the previous response, FDA concluded that foods irradiated in accordance with this regulation are safe on the basis of the findings and conclusions of BFIFC, the Task Group, and other information in the agency files.

#### 1. Consideration of Wholesomeness Studies

HEI stated that:

The FDA admits that "useful information has been learned from those feeding studies where there has been some exaggeration of dose relative to that prescribed by this regulation," and argues that such information establishes the safety of food irradiated in accordance with the regulation (51 FR 13382). In a review of 1,223 wholesomeness studies conducted by J. Barna for the Hungarian Academy of Sciences in 1979, study results were classified as either neutral, adverse, or beneficial. Each study could have several outcomes, since studies could address more

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Part 179

[Docket Nos. 86F-0507 and 86F-0509]

Irradiation in the Production, Processing and Handling of Food

AGENCY: Food and Drug Administration, HHS.

ACTION: Final rule; denial of request for stay of effective date and for a hearing; confirmation of effective date.

SUMMARY: The Food and Drug Administration (FDA) is denying the requests for a hearing that it has received on the final rule that amended the food additive regulations to authorize the use of sources of ionizing radiation for the control of food-borne pathogens in poultry. After reviewing the objections to the final rule and the requests for a hearing, the agency has concluded that the objections do not raise issues of material fact that justify a hearing or otherwise provide a basis for revoking the amendment to the regulation. FDA is also denying the request for a stay of the effective date of the amendment to the food additive regulations.

DATES: Effective date confirmed: May 2, 1990.

FOR FURTHER INFORMATION CONTACT: Patricia A. Hansen, Center for Food Safety and Applied Nutrition (HFS-206), Food and Drug Administration, 200 C St. SW., Washington, DC 20204, 202-418-3093.

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I. Introduction

In the Federal Register of May 2, 1990 (55 FR 18538), FDA issued a final rule permitting the use of ionizing radiation for the control of food-borne pathogens in poultry (the "poultry final rule"). This regulation, codified under 21 CFR 179.26, was issued in response to petitions filed by Radiation Technology, Inc. (RTI) (Docket No. 86F-0507), and the U.S. Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS) (Docket No. 86F-0509). In the Federal Register of March 3, 1987 (52 FR 6391), FDA published a notice announcing the filing of the petition submitted by RTI (FAP 8M3422), and in the Federal Register of February 20, 1987 (52 FR5343), FDA published a notice announcing the filing of the petition submitted by USDA, FSIS, (FAP 7M3974). FDA based its decision on data contained in both petitions and in its files.

II. Objections, Requests for a Hearing, and Request for a Stay

Section 409(f) of the Federal Food, Drug, and Cosmetic Act (the act) (21 U.S.C. 348(f)), provides that, within 30 days after publication of an order relating to a food additive regulation, any person adversely affected by such order may file objections, specifying with particularity the provisions of the order "deemed objectionable, stating reasonable grounds therefor," and may request a public hearing based upon such objections. FDA may deny a hearing request if the objections to the regulation do not raise genuine and substantial issues of fact that can be resolved at a hearing.

Under 21 CFR 171.110 of the food additive regulations, objections and requests for a hearing are governed by part 12 (21 CFR part 12) of FDA's regulations. Under § 12.22(a) each objection: (1) Must be submitted on or before the 30th day after the date of publication of the final rule; (2) must be separately numbered; (3) must specify with particularity the provision of the regulation or proposed order objected to; (4) on which a hearing is requested must specifically so state; failure to request a hearing on an objection constitutes a waiver of the right to a

hearing on that objection; and (5) requesting a hearing must include a detailed description and analysis of the factual information to be presented in support of the objection. Failure to include a description and analysis for an objection constitutes a waiver of the right to a hearing on that objection.

Following publication of the poultry final rule, FDA received several identical letters with multiple signatures and two submissions from Food and Water, Inc. (FWI), within the 30-day objection period. The submissions sought revocation of the final rule and requested a hearing. One of FWI's objections also requested that the regulation be stayed pending a public hearing of the scientific issues. The other FWI submission also requested an extension of the "comment" [sic] period.

III. Standards for Granting a Hearing

Specific criteria for deciding whether to grant or deny a request for a hearing are set out in § 12.24(b). Under the regulation, a hearing will be granted if the material submitted by the requester shows, among other things, that: (1) There is a genuine and substantial factual issue for resolution at a hearing; a hearing will not be granted on issues of policy or law; (2) the factual issue can be resolved by available and specifically identified reliable evidence; a hearing will not be granted on the basis of mere allegations or denials or general descriptions of positions and contentions; (3) the data and information submitted, if established at a hearing, would be adequate to justify resolution of the factual issue in the way sought by the requestor; a hearing will be denied if the data and information submitted are insufficient to justify the factual determination urged, even if accurate; and (4) resolution of the factual issue in the way sought by the person is adequate to justify the action requested; a hearing will not be granted on factual issues that are not determinative with respect to the action requested (e.g., if the action would be the same even if the factual issue were resolved in the way sought).

A party seeking a hearing is required to meet a "threshold burden of tendering evidence suggesting the need for a hearing" (Costle v. Pacific Legal Foundation, 445 U.S. 198, 214-215 (1980) reh. den., 445 U.S. 947 (1980), citing Weinberger v. Hynson, Westcott & Dunning, Inc., 412 U.S. 609, 620-621 (1973)). An allegation that a hearing is necessary to "sharpen the issues" or to "fully develop the facts" does not meet this test (Georgia Pacific Corp. v. U.S. E.P.A., 671 F.2d 1235, 1241 (9th Cir.

granting a hearing because a hearing request must include specifically identified reliable evidence that can lead to resolution of a factual issue in dispute. A hearing will not be granted on the basis of mere allegations or denials or general descriptions of positions and contentions (§ 12.24(b)(2)). Therefore, FDA is denying the hearing requested by these letters.

**b. Objections by FWI.** In one of its submissions, FWI contends that "FDA has failed to demonstrate that there is a 'reasonable certainty' that irradiation of poultry at 300 krad [3 kGy] is not harmful, and that therefore the Agency's approval is arbitrary and capricious." FWI gives four reasons for its contention.

**i. Power of the CIVO chronic rat feeding study.** First, FWI raises an issue about the statistical power of the chronic feeding study in rats conducted by CIVO. Specifically, FWI asserts that this feeding study was inadequate for determining safety because the study did not have sufficient statistical power to demonstrate that the cancer risk from consumption of irradiated chicken would be less than one in a million. FWI stated: "In accordance with procedures applied to food additives generally, testing must be of such sensitivity that even a small incremental risk of cancer cannot escape detection, namely one per million, extrapolated to a typical human consumer." FWI provided the results of statistical analyses regarding the power of the test. In a background statement in its submission, FWI also stated that "(g)iven the evidence that the formation of genotoxic radiolytic products can and does occur, a petitioner seeking approval of irradiation of poultry \* \* \* should bear the burden of establishing the magnitude of expected cancer risk, or that it is below a stated level." In support of its objection, FWI submitted only a table entitled "Identification of Genotoxic Radiolytic Products in Irradiated Organic Media or Food," but this table contained no information on genotoxicity data from irradiated poultry. FWI's objection did not dispute FDA's conclusion that the evidence demonstrated that irradiated poultry was not mutagenic (55 FR 18538 at 18540).

Neither FDA's guidelines nor generally accepted scientific procedures suggested for food additive testing recommend that carcinogenicity testing be sufficiently sensitive to detect an increased cancer risk of one in one

million.<sup>2</sup> FWI provided no information to support its contention, either by reference to FDA's regulations or to any other requirement. Thus, FDA concludes that this objection raises no issue of fact that can be resolved at a hearing. Instead, the objection simply states FWI's preference for a policy regarding carcinogenicity testing. A hearing will not be granted on issues of policy or law (§ 12.24(b)(1)).

In addition, FDA does not dispute FWI's contention that the statistical power of this test is such that it cannot detect an increased cancer risk of one in one million. However, FWI did not demonstrate why prevailing on this factual issue would be adequate to justify the action requested (§ 12.24(b)(4)).

Additionally, FWI suggested that to increase sensitivity of the testing the radiation dose should have been increased tenfold or that concentrated extracts of all radiolytic products formed by irradiating chicken should have been fed.<sup>3</sup> Once again, FWI

<sup>2</sup> In fact, it would not be feasible to conduct such testing in laboratory animals for substances ordinarily consumed at anything other than trivially low levels in the diet. Generally, to increase the power of a test one must increase the amount of test substance fed or increase the number of animals in each group. For example, the standard approach to assess low levels of carcinogenic risk is to feed a substance in large amounts, determine the risk at such a high dose, and extrapolate to lower doses using a linear extrapolation model. Using such a model to detect an increased risk of one in one million from a substance and assuming that the study design could detect a 10 percent cancer incidence at a high dose, one would have to feed an animal 100,000 times the amount it would consume under realistic conditions. This clearly cannot be done with a diet of chicken. Alternatively, testing thousands of animals per group would overwhelm normal laboratory capabilities.

Under FDA guidelines, testing of a food additive is generally conducted at levels no higher than 5 percent of the diet for nonnutritive substances. This level can be higher for a nutritive substance, however, provided it does not cause a significant nutritional deficit (Ref. 1). As noted previously and discussed in detail in the poultry final rule, the CIVO studies fed chicken irradiated at the maximum dose allowed by the regulation, as well as at twice that dose, in amounts equivalent to 35 percent of the diet (by dry weight). Moreover, based on its review of the mutagenicity data, FDA concluded that there was no basis to suspect that irradiated chicken would be carcinogenic.

<sup>3</sup> Irradiation doses typically can be raised only marginally higher than would be used in practice before they produce effects that would change food significantly, often producing an unpalatable product that animals will not eat. Special processing conditions can be used to minimize such effects, however, such as irradiating food in the frozen state in the absence of air. In the poultry final rule, FDA cited tests conducted at a dose approximately 10 times higher than the CIVO studies, which studies showed no adverse effects related to irradiation (55 FR 18539 at 18540). FDA relied primarily on the CIVO studies, however, because FDA would not expect irradiation of poultry at a dose below 3 kGy to be conducted

submitted no information to establish that the testing it recommended is required to demonstrate safety, or even that such testing would be valid to assess safety. Nor did FWI provide any information concerning how one can conduct such a study or how one can interpret the findings in the context of poultry irradiated at a dose not to exceed 3 kGy. Because FWI provided no evidence to consider in support of its assertion, FDA is denying the request for a hearing on this point because a hearing will not be granted on the basis of mere allegations or denials or general descriptions of positions and contentions (§ 12.24(b)(2)).

**ii. Addition of ethoxyquin to irradiated chicken in the CIVO studies.**

In the CIVO studies, the researchers removed water from the chicken by drying over hot air, in order to preserve the chicken for the time needed to complete the testing. Prolonged contact with hot air causes lipids (fats) to be oxidized to lipid peroxides, thereby rendering the food rancid and unpalatable. Prolonged storage can also lead to rancidity. Thus, the researchers added ethoxyquin, an antioxidant, to the chicken to prevent rancidity. Preventing rancidity by this means is of importance for a product dried and stored, as in the test.

In its second contention, FWI states that the CIVO studies were seriously compromised because the addition of the antioxidant ethoxyquin to the chicken decreased the levels of lipid peroxides in the irradiated chicken to levels comparable to those in unirradiated chicken. FWI contends that these decreased levels would interfere with the observation of toxicity from the lipid peroxides that were formed in higher amounts during the hot air drying of irradiated chicken than in the unirradiated chicken.

In the poultry final rule, FDA noted that ethoxyquin had been incorporated into both the control diets and the test diets in the CIVO studies. The agency acknowledged (55 FR 18538 at 15839 and 15840) that FDA reviews of the CIVO studies had raised the question of

using the processing conditions required for the higher dose.

Extracts of irradiated foods have not been relied on primarily for testing because radiolytic products of food do not differ in any particular chemical or physical properties from other components of food that would allow them to be specifically extracted from food. Additionally, radiolytic products are typically identical to substances that occur naturally in foods. Therefore, FDA is not aware of how one could prepare an extract that would ensure the presence of all radiolytic products while excluding the presence of other similar components of food that did not result from irradiation. The only way to ensure that all radiolytic products are present is to feed the irradiated food itself.