Chapter 4

TOXICOLOGICAL SAFETY
OF IRRADIATED FOODS

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Introduction

Generation of cancers in animals requires the mutation or deletion of oncogenes or tumor suppressor genes, resulting in a loss of heterozygosity at those allele locations. Mutation (point mutations or frame-shift mutations) and deletion of genes can be induced by exposure of cells to genotoxic chemicals or can occur naturally as part of the cellular DNA repair and replication process (Ames and Gold 1990).

Many consumers are simply unaware that foods contain carcinogens, either natural or artificial, and cause cancer. A very small subset of naturally occurring carcinogens in foods include compounds such as benzene and formaldehyde (Smith and Pillai 2004; Fan and Thayer 2002). A number of studies have confirmed the mutagenicity of cooked meats and their fats, and the formation of nitrosamines as part of the meat curing and cooking process (Knekt and others 1999). Tumor promoters present in cooked meat and poultry include oxidation products of fats and oils, heme, and cholesterol (Van der Meer-van Kraaij and others 2005; Yang and others 1998; Tseng 1996). Alcohol is known to induce the formation of tumors in the gastrointestinal tract of rodents (Mufti 1998).

It was recently found that high-temperature frying and baking of starch-containing foods results in the formation of acrylamide, a suspected human carcinogen (Friedman 2003). Furan, a carcinogen in animals, is formed in foods as a result of thermal processing (Perez-Locas and Yaylayan 2004). Compounds used in the pickling, salting, and smoking processes are associated with gastro-intestinal cancers in humans (Weisburger and Jones 1990). Discussions pertaining to food irradiation
therefore have to be placed in context with the risks associated with consumption of irradiated foods versus foods processed using technologies and additives that are known to cause cancer in animals and humans.

**Food Irradiation**

Food irradiation is perhaps the single most studied food processing technology for toxicological safety in the history of food preservation. Studies pertaining to the safety and nutritional adequacy of irradiated foods date back to the 1950s and were frequently associated with the use of radiation to sterilize foods. Hundreds of short-term and long-term safety studies led to the approval of one or more foods for irradiation by presently more than sixty countries. These studies are thoroughly reviewed in *The Safety and Nutritional Adequacy of Irradiated Foods*, published by the World Health Organization (WHO 1994).

In the United States, the Food and Drug Administration reviewed the available studies for the quality of experimental design, rigor, and statistical validity before approving irradiation of a variety of food products including grain, fruits and vegetables, spices and dried herbs, meat and poultry, and eggs for human consumption (Federal Register 2005; WHO 1994). The vast majority of the studies failed to find adverse effects associated with consumption of or exposure to irradiated foods. Not surprisingly, a small number of studies produced equivocal results pertaining to the safety of irradiated foods. However, in-depth review of those studies determined that they were deficient in experimental design, used insufficient numbers of test subjects for proper statistical analysis, or suffered from experimenter error (WHO 1994).

The preferred method for assessing the toxicological safety of irradiated foods has been long-term feeding studies in animals, often for multiple generations. Toxicologists prefer to use animals for these types of evaluations, as opposed to using people or their children, for obvious reasons. Swallow (1991) reported that animals used for toxicological research, fed diets of radiation-sterilized foods for 40 generations, suffered no ill effects from consumption of the irradiated foods. Thayer and others (1987) reported that rodents fed diets of radiation-sterilized chicken meat (45–68 kGy) did not suffer an increased risk of cancer or birth defects. The same study also failed to find adverse affects associated with long-term consumption of irradiated meat in beagle dogs. De Knecht-van Eekelen and others (1971, 1972) conducted single- and multiple-generation feeding studies in rats without finding adverse effects due to consumption of the irradiated chicken diet. Poling and others (1955) re-
ported no evidence of changes in survival, histopathology, or reproduction in three generations of rats fed radiation-sterilized ground beef. Feeding studies in animals have been very consistent in the lack of adverse effects associated with long-term consumption of irradiated foods.

**Benzene, Formaldehyde and Amines**

The presence of several compounds, most notably benzene and toluene, has generated some concerns about the safety of irradiated foods. It was originally thought that trace amounts of benzene were formed in irradiated foods and that this was a unique situation.

It is currently thought that benzene and toluene are produced from the oxidative/radiolytic cleavage of phenylalanine. They have been reported in irradiated beef (Merritt and others 1978; Nam and others 2003), though in the 5–60 ppb range. Benzene and its derivatives are not typically found in raw food products, but it appears that thermal treatments do produce trace amounts in some cooked products. Matiella and Hsieh (1991) identified benzene derivatives in scrambled eggs, whereas McNeal and others (1993) reported the presence of benzene in butter, eggs, meat, and certain fruits with levels ranging from 0.5 ppb in butter to 500-1900 ppb in eggs. Angelini and others (1975) evaluated volatile compounds in fresh and irradiated haddock and found benzene and toluene in all samples with larger quantities present in the irradiated ones.

In 1979 the Federation of American Societies for Experimental Biology evaluated 65 compounds found in irradiated beef and noted that small amounts of benzene could be detected in both irradiated (56 kGy) and untreated beef (Chinn 1979a). Gamma and electron-irradiated beef contained about 18–19 ppb, which was reduced to 15 ppb upon cooking. On the other hand, the thermally-sterilized and frozen controls contained no detectable benzene, but on cooking the levels were approximately 2–3 ppb. They concluded that such small amounts of benzene did not constitute a significant risk.

Health Canada (Bureau of Chemical Safety), in a recent evaluation (2002) of an application for irradiated ground beef, has estimated that approximately 3 ppb of benzene would be formed in irradiated beef at the typical dose ranges (1.5–4.5 kGy) and concluded that it is of insignificant health risk.

Formaldehyde and malonaldehyde are probably formed in most foods containing carbohydrates (Dauphin and Saint Lebe 1977). Usually the formed formaldehyde is very reactive and will readily form covalent links with proteins and other constituents. Thus, unless the food item is low in
protein or contains a considerable amount of water, it would not be present. Fan (2003) has shown that formaldehyde can be generated from solutions of fructose, glucose, and sucrose. Significant amounts were also observed in both pasteurized and fresh irradiated apple juice (Fan and Thayer 2002). Lee and others (1973) observed slight increases in formaldehyde amounts in irradiated Irish Cobbler potatoes at doses of 1.5 kGy.

Irradiation has been suggested as a way to control nitrosamine formation in cured meat products such as bacon (Fiddler and others 1985). Ahn and others (2004), using water solutions, have shown that the nitrosamines, nitrosodimethylamine, and nitrosopyrrolidine were significantly reduced by gamma irradiation in addition to residual nitrite levels. However, the reduction was not apparent unless the irradiation dose was 10 kGy or higher, an unrealistically high dose. Similar results were observed when using irradiated cooked pork sausage where doses of 5 or 10 kGy reduced residual nitrite levels and nitrosodimethylamine and nitrosopyrrolidine (Ahn 2004). Thus it appears that irradiation can destroy preformed nitrosamines directly or, by limiting residual nitrite or reactive nitrogen compounds, can inhibit the formation upon cooking.

Use of irradiation to reduce other toxic nitrogenous compounds, the biogenic amines, has been evaluated in fermented soybean paste. Irradiation of the paste prior to fermentation did not produce any differences compared to controls. After fermentation for 12 weeks, there were significantly lower amounts of histamine, putrescine, tryptamine, and spermidine in the treated samples, suggesting that irradiation may have altered the microflora to one not conducive to biogenic amine formation (Kim and others 2003). Levels of the biogenic amines putrescine, tryptamine, spermine, and spermidine were reduced in pepperoni subjected to gamma irradiation (5–20 kGy) prior to storage (4° C, 4 wk), again suggesting reduction in bacterial numbers (Kim and others 2005).

**Formation and Levels of 2-ACBs in Foods**

Particular attention has been drawn to a special class of cyclic compounds being formed on irradiation of lipids. A wealth of radiolytic products are formed on irradiation of, for example, triglycerides, among them fatty acids, hydrocarbons, aldehydes, ketones, esters, and dimeric and polymeric components (Nawar 1978, 1986; Stewart 2001), but to date one class of components, the 2-alkylcyclobutanones (2-ACBs), is of particular interest. This new class of cyclic components was reported more than 30 years ago (LeTellier and Nawar 1972) to be formed on irradiation
of pure saturated triglycerides containing \( C_6 \), \( C_8 \), \( C_{10} \), \( C_{12} \), \( C_{14} \), \( C_{16} \), and \( C_{18} \) fatty acids with a high dose (60 kGy under vacuum). These compounds were identified as the 2-alkylcyclobutanones of the same carbon number as the precursor fatty acid. It has been proposed that these compounds may result from cleavage of the acyl-oxy bond via the formation of a six-membered ring intermediate (Figure 4.1).

2-Dodecylcyclobutanone (2-dDCB) derived from palmitic acid (\( C_{16} \)) was also identified in an irradiated synthetic phospholipid, that is, di-palmitoyl-phosphatidyl-ethanolamine, which had been treated with a very high radiation dose (500 kGy under air) (Handel and Nawar 1981). It was, however, not before 1990 that a 2-ACB was identified in irradiated food. Stevenson and others (1990) reported the detection of 2-dDCB in chicken irradiated at a dose of 5 kGy. Subsequent work indicated that the 2-ACBs are radiation specific because they are not detected in raw, cooked, frozen, freeze-dried, spoiled chicken, thermally sterilized chicken stored at room temperature for 12–13 years, or chicken exposed to modified atmospheres (Boyd and others 1991; Crone and others 1992a, b; Stevenson and others 1993).

In another approach Ndiaye and others (1999a) treated an aqueous suspension of a synthetic mixture of pure saturated triglycerides (\( C_{10} \), \( C_{12} \), \( C_{14} \), \( C_{16} \) and \( C_{18} \)) with microwaves (20 min at 750 W, 2450 MHz),

\begin{figure}
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\caption{Formation of 2-ACB on irradiation of a triglyceride (LeTellier and Nawar 1972; Nawar 1978, 1986; see also Stewart 2001).}
\end{figure}
with heat in a convection oven (30 min at 150° C), with UV irradiation (60 min with \( \lambda \) 240–280 nm), with high pressure (60 min with 6000 bar) and with ultrasound (5 min at 455 W, 20 kHz) and were unable to detect any 2-ACBs. However, if the triglyceride solution was irradiated, all the corresponding 2-ACBs could be identified. Thus every fatty acid gives rise to its own 2-ACB (Table 4.1).

To date, 2-ACBs have not been identified in nonirradiated foods. An exotic occurrence may be the possible presence of 2-methylcyclobutanone in *Hevea brasiliensis* (Nishimura and others 1977). These authors speculated that the cyclization of isoprene components after sonication could lead to the cyclobutanone structure, but as mentioned previously, when saturated triglycerides were treated with sonication, no 2-ACBs could be detected (Ndiaye and others 1999a). This seems to support the hypothesis that 2-ACBs may be radiation specific, thus being “Unique Radiolytic Products.” However, the possibility also exists that available detection methods of 2-ACBs are just not sensitive enough, so the amount possibly present also in non-irradiated foods is at the moment below the detection limit of the analytical methods (Ndiaye and others 1999a).

The occurrence of 2-ACBs in many irradiated foodstuffs has now been confirmed in meat (beef, pork, lamb), poultry (chicken, mechanically re-

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>2-alkylcyclobutanone</th>
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<tr>
<td>C 10:0 Capric acid</td>
<td>2-hexyl-cyclobutanone (2-HCB)</td>
</tr>
<tr>
<td>C 12:0 Lauric acid</td>
<td>2-octyl-cyclobutanone (2-OCB)</td>
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<tr>
<td>C 14:0 Myristic acid</td>
<td>2-decyl-cyclobutanone (2-DCB)</td>
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<tr>
<td>C 16:0 Palmitic acid</td>
<td>2-dodecyl-cyclobutanone (2-dDCB)</td>
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<tr>
<td>C 16:1 Palmitoleic acid</td>
<td>2-(dodec-5′-enyl)-cyclobutanone (2-dDeCB)</td>
</tr>
<tr>
<td>C 18:0 Stearic acid</td>
<td>2-tetradecyl-cyclobutanone (2-tDCB)</td>
</tr>
<tr>
<td>C 18:1 Oleic acid</td>
<td>2-(tetradec-5′-enyl)-cyclobutanone (2-tDeCB)</td>
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<tr>
<td>C 18:2 Linoleic acid</td>
<td>2-(tetradeca-5′,8′-dienyl)-cyclobutanone (2-tD2eCB)</td>
</tr>
<tr>
<td>C 18:2 Linolenic acid</td>
<td>2-(tetradeca-5′,8′,11′-triienyl)-cyclobutanone (2-tD3eCB)</td>
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covered poultry meat), liquid whole egg, cheese (Camembert, Brie, or cheese made from sheep’s milk), seafood (prawns), fish (sardine, trout, salmon), fruit (mango, papaya, avocado), nut (peanut), seeds (perilla), and cereals (rice) (see references in EN 1785:2003). 2-dDCB could be also identified in foods irradiated at very low doses (0.05–0.1 kGy) such as onions, garlic, rice, or cowpeas (Horvatovich and others 2002a; Ndiaye 1998; Ndiaye and others 1999b).

The levels of 2-ACBs seem to vary in different foods. Of course, this depends on the fat content of the food, its fatty acid composition, and the dose of radiation. Also, the irradiation temperature plays a role, less 2-ACBs being formed in frozen foodstuffs. Generally a linear dose dependency of the formation of 2-ACBs has been observed (Stevenson 1992, 1994; Stevenson and others 1993; Crone and others 1992a, b; Ndiaye and others 1999a; Park and others 2001; Tanabe and others 2001; Gadgil and others 2002; Burnouf and others 2002).

Mostly, the level of 2-ACBs is given per gram of fat and is reflective of the fatty acid composition of the meat. For chicken meat, values between 0.15 and 0.75 µg 2-dDCB / g lipid / kGy have been reported (Stevenson and others 1992, 1993, 1996; Boyd and others 1991; Crone and others 1992a, b; Ndiaye and others 1999a; Stewart and others 2001; Tanabe and others 2001), whereas values for 2-tDCB were about 0.05–0.1 µg / g lipid / kGy. The levels in pork meat varied from 0.13–0.21 µg 2-dDCB / g lipid / kGy and 0.12–0.27 µg 2-tDCB / g lipid / kGy (Stevenson 1994, 1996; Stewart and others 2001; Park and others 2001). For beef the reported 2-dDCB yield was about 0.1–0.18 µg / g lipid / kGy, and for 2-tDCB it amounted to be ~ 0.14 µg / g lipid / kGy (Stevenson 1994; Gadgil and others 2002).

A systematic approach was taken by Ndiaye and others (1999a), who related the amount of 2-ACBs to the precursor fatty acid. For several foodstuffs (cheese, sardine, trout, beef, and poultry meat) irradiated between 0.1 and 3.1 kGy, the yield of saturated 2-ACBs was reported to be between 1.0 and 1.6, in average 1.3 ± 0.2 nmol / mmol precursor fatty acid / kGy. This indicated that the amount of 2-ACBs (in this case, saturated 2-ACBs) formed is relatively independent of the food or food matrix and mostly reflects the amount of precursor fatty acid. This was confirmed by Burnouf and others (2002), who irradiated various types of foods, that is, milk powder, hazelnuts, chicken, beef, goose liver, cocoa, ground beef patties, smoked salmon, frog legs, chicken quenelles, salmon, avocado, liquid whole egg, and noticed that in general similar yields expressed in nmol 2-ACBs / mmol precursor fatty acid / kGy were obtained, although some variation was obvious. This variation could possibly be ascribed to different positions of the fatty acids in the triglycerides (Stevenson 1994). In a
recent study Horvatovich and others (2005) reported that the formation of saturated 2-ACBs (2-dDCB, 2-tDCB) in chicken, liquid whole egg, and avocado was 1.4 ± 0.4 nmol / mmol precursor fatty acid / kGy. For the formation of mono-unsaturated 2-ACBs (cis-2-dDeCB, cis-2-tDeCB) from the same foods, a slightly lower value of 0.9 ± 0.3 nmol / mmol precursor fatty acid / kGy was found, although this value was found not to be significantly different from the value for the saturated 2-ACBs (Horvatovich and others 2005).

In a study with pure triacylglycerides (C_{16}, C_{18}, C_{18:1}, C_{18:2}, C_{18:3}) and the corresponding authentic fatty acids, different radioproduction yields were reported (Kim and others 2004), with the highest levels of 2-ACBs being observed for the saturated triglycerides.

In food with mixed triglycerides and usually low amounts of free fatty acids, phospholipids, sterols and other fat components, the radioproduction levels seem to vary only slightly (Ndiaye and others 1999a; Burnouf and others 2002; Horvatovich and others 2005). Therefore, if the fat composition of the food sample is known, the levels of 2-ACBs can be roughly predicted. Considering the edible fat containing only triglycerides and restricting this rough calculation only to the four most common fatty acids in food, namely palmitic acid, stearic acid, oleic acid, and linoleic acid, and taking into account the most recent formation factors of Horvatovich and others (2005) of 1.4 nmol for the saturated 2-ACBs and 0.9 nmol for the unsaturated 2-ACBs per mmol precursor fatty acid per kGy—as a first approximation the formation factor of the di-unsaturated 2-ACB from linoleic acid is set to be similar to that of the mono-unsaturated 2-ACB from oleic acid—the prediction leads to the following yields: for chicken meat containing about 12.5% edible fat with a composition of approx. 21% palmitic acid, 6% stearic acid, 32% oleic acid, and 25% linoleic acid, which has been irradiated at the maximum dose of 3 kGy, levels of 2-ACBs amounts to about 11 µg 2-dDCB, 3 µg 2-tDCB, 10 µg 2-tDeCB, and 8 µg 2-tD2eCB per 100 g of fresh irradiated (3 kGy) chicken.

For beef, for example, ground beef patties containing a maximum of 23% fat with a fatty acid composition of approx. 27% palmitic acid, 15% stearic acid, 43% oleic acid, and 3.8% linoleic acid, a similar calculation arrives at 36 µg 2-dDCB, 20 µg 2-tDCB, 37 µg 2-tDeCB, and 3 µg 2-tD2eCB per 100 g of fresh irradiated (maximum dose 4.5 kGy) beef.

If a 20% loss in 2-ACBs after cooking (Crone and others 1992a) is anticipated and the actual mean intake of poultry (as given by Health Canada 2003) is about 62.1 g poultry per day, this intake would provide 0.08 µg 2-dDCB + 0.02 µg 2-tDCB + 0.08 µg 2-tDeCB + 0.06 µg 2-tD2eCB per kg body weight (kg bw) per day. This makes a total intake due to irradiated poultry of about 0.24 µg 2-ACBs / kg bw / day.
Similar for beef with a daily intake of 23.2 g (Health Canada 2003), the consumption of irradiated beef would result in an intake of 0.10 µg 2-dDCB + 0.06 µg 2-tDCB + 0.10 µg 2-tDeCB + 0.01 µg 2-tD2eCB per kg body weight per day. The total intake of 2-ACBs due to irradiated beef would thus amount to 0.27 µg 2-ACBs / kg bw / day.

The daily intake by irradiated beef and poultry results in a value of 0.51 µg 2-ACBs / kg bw / day. Of course it should be taken into account that not all beef and poultry at present is being irradiated, and this can also not be expected in the near future. Only a very low percentage of beef and poultry is presently irradiated. The rough calculation shows a conservative value, which, however, could increase if higher radiation doses used for sterilization were to be applied and if other irradiated fat-containing foodstuffs with considerable amounts of 2-ACBs would be consumed.

This calculated daily intake of roughly 0.5 µg 2-ACBs / kg bw may be compared with the estimated uptake of acrylamide by, for example, fried food such as french-fried potatoes or potato crisps, the average value for the general population reported by the WHO (2002) being about ~ 0.3–0.8 µg acrylamide / kg bw / day. However, the toxicology database of acrylamide contains much information, whereas knowledge about the toxicological properties of 2-ACBs is still scarce. Already during the evaluation of the health aspects of certain compounds found in irradiated beef in the 1970s by FASEB, it was mentioned that metabolic and toxicological studies of the 2-ACBs presumably present in beef would be desirable (Chinn 1979b). At that time, 2-ACBs had not yet been identified in food, but only in triglycerides irradiated at high doses (60 kGy).

The total daily intake of roughly 40 µg 2-ACBs per person per day surpasses the recently discussed threshold of toxicological concern (TTC) of 1.5 µg / person / day (Barlow and others 2001), which is used by the U.S. FDA for reviewing components of food contact materials with low exposures. So it may be prudent to collect more knowledge on the toxicological and metabolic properties of 2-ACBs in order to quantify a possible risk—albeit minimal.

Knowledge about the metabolism of 2-ACBs is very restricted. Only one study about the fate of 2-ACBs in rats has been published (Horvatich and others 2002b). Rats received a drinking fluid containing 0.005% 2-tDCB or 2-tDeCB daily for four months. The 2-ACBs could be identified in very low amounts in the adipose tissues of the rats (10^5 times the total quantity consumed). Less than 1% of the 2-ACBs ingested daily were excreted in the faeces. These results indicate that 2-ACBs are probably largely metabolized. Thus, further metabolic studies are desirable.
Toxicological Safety of 2-ACBs

Although the toxicological safety of irradiated meat and poultry has been studied extensively, far less data is available pertaining to the genotoxic potential of 2-ACBs, the chemicals that are formed by the radiolysis of triglycerides, phospholipids, and fatty acids. Controversy over the genotoxicity of the 2-ACBs started following the publication of preliminary data by Delincée and Pool-Zobel (1998) in which 2-dDCB at concentrations of 0.30–1.25 mg / ml in in vitro experiments induced DNA strand breakage in primary human and rodent colon cells using the Comet Assay. The authors’ study cautioned against interpretation of the results to infer that irradiated foods were carcinogenic and instead called for more study on the issue of 2-ACB genotoxicity (Delincée and Pool-Zobel 1998). The Comet Assay, although used extensively as a screening assay, has not been validated for the detection of weak genotoxins and can produce false-positive results due to the chromosome degradation that occurs as a result of non-genotoxic cell death (Health Canada 2003; Tice and others 2000). A retest of multiple 2-ACBs in the Comet Assay, in human HT-29 and HT-29 cl 19A cells at concentrations up to 400 µM, failed to detect significant levels of DNA strand breakage (Burnouf and others 2002).

The genotoxicity of 2-ACBs was also studied in two human cell lines, HeLa and HT-29, using an alkaline unwinding procedure to quantify DNA strand breaks and Fpg-sensitive sites following the procedure proposed by Hartwig and others (1996). The frequencies of both DNA strand breaks and oxidative DNA modifications served as sensitive indicators of DNA damage. The results obtained thus far demonstrate that all of the test compounds that were investigated (2-tDCB, 2-tDeCB, 2-dDCB and 2-DCB) have cytotoxic effects in both cell lines at concentrations ≥100 µM. All of the 2-ACBs were also shown to induce oxidative DNA damage. In the case of 2-tDCB and 2-tDeCB, DNA damage occurred only at concentrations that were already highly cytotoxic, such that considerable fractions of the cells were no longer viable. The situation was different with 2-dDCB and 2-DCB, where oxidative DNA damage occurred at non-cytotoxic concentrations, making these results more relevant to the toxicological assessment (Burnouf and others 2002; Marchioni and others, 2004).

Several 2-ACBs including 2-dDCB have also been tested in the Salmonella Mutagenicity Test (SMT), with no induction of mutations due to exposure to 2-ACBs using TA97, TA98, and TA100 tester strains being detected (Burnouf and others 2002). Other laboratories have focused their efforts on 2-dDCB, a prevalent 2-ACB in ground beef formed by the radiolysis of palmitic acid. Two studies investigated the ability of 2-dDCB to induce mutagenesis in the bacterial reverse mutation assays, with and
without exogenous metabolic activation (Sommers 2003; Sommers and Schiestl 2004). No increase in the formation of mutants was observed in the SMT or the E.coli TRP Assay using tester strains WP2 [pKM101], WP2 uvrA [pKM101], TA98, TA100, TA1535, and 1537, which were in agreement with results published by Burnouf and others (2002). Gadgil and Smith (2004) also investigated the ability of 2-dDCB to induce mutations in the SMT using tester strains TA97, TA98, TA100, TA102, and TA1535, and failed to detect an increase in the formation of mutants as a result of 2-dDCB exposures up to 1 mg / plate. Three laboratories have now failed to detect an increase in mutagenesis as a result of exposure to 2-dDCB, or multiple 2-ACBs, in the widely used and validated SMT and E. coli TRP Assays.

In forward mutagenesis assays, an entire gene is a target for mutagenesis, as opposed to single nucleotide changes that are detected in the bacterial reversion tests. 5-Fluorouracil (5-FU)-resistant mutants in E. coli or Salmonella are formed when a null mutation is fixed within the DNA sequence of the 0.551 kb uracil-phosphoribosyltransferase gene, which would normally convert 5-FU to a toxic metabolite within the bacterium (Skopek and Thilly 1983). Sommers and Mackay (2005) failed to detect an increase in the formation of 5-FU mutants in E.coli following exposure to 1 mg / ml 5-FU, with or without exogenous metabolic activation.

Gene expression profiling has also been used extensively for determination of genotoxic potential and is capable of identifying many genotoxins that are not detectable using bacterial reverse mutation assays. Transcription of RNA from the DNA damage-inducible UmuDC, RecA, DinD, and Nfo DNA genes of E. coli has been shown to increase following exposure to genotoxins (Orser and others 1995a, b). 2-dDCB was not able to induce gene expression from any of those gene promoters, as measured by increased β-galactosidase activity levels, at concentrations up to 1 mg / mL in E.coli SF1 containing each of the aforementioned promoter/β-galactosidase reporter constructs, with or without exogenous metabolic activation (Sommers and Mackay 2005). This is in contrast to other carcinogens routinely present in foods including formaldehyde, dimethylnitrosamine, and aflatoxin B1 as shown by Orser and others (1995a, b).

The Microtox™ system uses the bioluminescent marine microorganism Vibrio fischeri to measure the acute toxicity of chemicals or environmental samples, and has been commercially available since the 1980s as a primary toxicity screen. Gadgil and Smith (2004) examined the cytotoxicity of 2-dDCB in the Microtox Assay in order to make a comparative analysis between 2-dDCB and common GRAS food additives including the carbonyl compounds cyclohexanone and 2-nonenal. In the Gadgil
and Smith (2004) study the EC_{50} values, the test compound concentrations that produced a 50% decrease in bioluminescence, were 21.7 ppm for 2-dDCB, 37.4 ppm for cyclohexanone, and 1.65 ppm for nonenal. The authors concluded that the acute toxicity of 2-dDCB was between that of carbonyl group containing GRAS food additives cyclohexanone and 2-nonenal in the Microtox Assay. These results are conflicting with those of Burnouf and others (2002), who in growth inhibition studies with *Salmonella typhimurium* bacteria found clear cytotoxic effects for several 2-ACBs, particularly for 2-DCB. The toxic dose (37% survival) of 2-dDCB was about 40 µM, but that of 2-DCB was only 4 µM (Marchioni and others 2004).

In addition to tests in bacteria, 2-dDCB has also been tested for the ability to induce rearrangement of chromosomes in eukaryotic cells. The Yeast (*Saccharomyces cerevisiae*) DEL Assay measures a compound’s ability to cause genomic rearrangements, induced by DNA strand breakage, by restoration of a nonfunctional duplication of the *his3* gene to functionality (*HIS3*) by intrachromosomal (DEL) recombination (Sommers and Schiestl 2004). The assay does not produce false positives due to cell death because only recombination events in live cells are selected for. This is unlike the Comet Assay, which detects only the DNA strand break, not the actual genetic endpoint. Concentrations up to 5 mg/ml of 2-dDCB, which reduced cell viability to 28%, failed to induce genomic rearrangements in the Yeast DEL Assay (Sommers and Schiestl 2004). In contrast, carcinogens commonly present in food such as benzene and formaldehyde each induce increases in intrachromosomal recombination in the yeast-based test (Sommers and others, 1995). Very recent experiments using Comet Assay to measure DNA strand breaks and 24-color-Fluorescence-In-Situ-Hybridization to estimate chromosomal abnormalities indicated that 2-dDCB had a genotoxic potential and caused chromosomal aberrations in human colon cells (Knoll and others 2005).

### 2-ACBs and Tumor Promotion

There have been very few studies on the ability of highly purified 2-ACBs to induce tumors in animals. Raul and others (2002) investigated the ability of 2-tDeCB and 2-tDCB to induce pre-neoplastic lesions (aberrant crypt foci) and tumors in the colons of Wistar rats. In that study, rats were fed 1% ethanol in water, or fed 1.6 mg/day 2-ACBs (about 6 mg / kg bw) dissolved in water that contained 1% ethanol as the 2-ACB solvent. The rats in each group were injected (intra-peritoneal) at weeks 2 and 3 with carcinogen azoxymethane (15 mg / kg bw), which induces pre-
neoplastic lesions (ACFs) and tumors in the colons of rodents. The animals were sacrificed at 3 and 6 months and the colons examined for the total number of aberrant crypt foci, the number of crypts per foci, and actual tumor formation. Only a small number of rats, six per group, were used in the study. For each of the test groups, the number of ACF/cm in the distal colon were similar, with no difference in the total number of ACFs being evident. However, a statistically significant, but less than twofold, increase in the number of aberrant crypts per foci was observed in the 2-tDeCB-treated rats after six months, but not after three months. After six months, the total number of tumors in the colon was threefold higher in the 2-ACB-treated animals than in the AOM controls. The colons of four of six AOM-control rats exhibited only one small tumor (~6 mm³). Multiple tumors were observed in four and three of six animals treated with 2-tDCB or 2-tDeCB, respectively, whereas medium (6 < S < 25 mm³) and larger (>25 mm³) tumors were detected only in 2-ACB-treated animals.

The possibility that one or more of the 2-ACBs at pharmacological doses could be tumor promoters prompted the authors to recommend further research into the tumor promotion phenomenon. Additional in vivo studies, using larger numbers of animals, with 2-ACBs incorporated into the feed of animals as opposed to drinking water, that use multiple 2-ACB concentrations are clearly warranted in order to more accurately assess the tumor-promoting potential of the 2-ACBs.

**Diet and Tumor Promotion**

Although the tumor-promoting potential of the 2-ACBs has not been fully elucidated, the increases in the number of aberrant crypts and tumors in 2-ACB treated animals that received large doses of the carcinogen azoxymethane and the tumor promoter ethanol would not be totally unexpected. Raul and others (2002) speculated that the increase in the number of aberrant crypts observed in their study might be due to the interaction of the fatty acid derivatives with the epithelial cells of the colon. The impact of high levels of dietary fat and the risk of chronic disease, including colon cancer, have been well documented (Weisburger 1997). Udilova and others (2003) found that dietary oil components can induce oxidative stress, lipid peroxidation in membranes, cytotoxicity, and enhanced risk of colon cancer through regenerative cell proliferation. Oxidized beef fat has been shown to induce the formation of colon tumors in rodents (Yang and others 1998). Other colon tumor promoters found in meat and poultry products include oxidized heme, cholesterol, and cholic acid (Van der Meer-van Kraaij and others 2005; Yang and...
others 1998; Tseng 1996). Consumption of high concentrations of fat and fat derivatives causes formation of tumors in the colons of rodents. It is not surprising, therefore, that large doses of purified 2-ACBs might induce formation of tumors in the colons of rodents.

Conclusions

Cancer in animals and humans has been associated with many factors including excessive consumption of fried, smoked, and barbecued meats and fish, pickled foods, and alcohol. Carcinogens such as formaldehyde, furan, acrylamide, nitrosamines, and benzene are naturally occurring in many foods, or formed as a result of thermal processing. Tumor promoters present (at milligram and gram quantities) in meat include lipids and oxidized lipids, hemes, and cholesterol. Because levels of 2-ACBs are present in sufficient (albeit µg) quantities to be considered an indirect food additive, assessment of their toxicological potential should be a priority in the science of food irradiation. It should also be recommended that any toxicological risk assessment pertaining to the 2-ACBs should be in the context of the total human diet and the potential benefit of food irradiation in reducing illnesses, hospitalizations, and deaths associated with foodborne illness.

Paracelsus, the fifteenth-century philosopher and scientist, observed that all substances are poisons; it is only a matter of dose. Although it is almost impossible to prove the absolute safety of any food or food processing technology, it is difficult to conceive—considering the toxicological database—that radiation-pasteurized foods, including meat and poultry, pose a significant risk to human health when consumed as part of a healthful, well-balanced diet. This is true especially when compared to other, “more established” food processing and preservation methodologies that have been directly associated with the formation of cancers in animals and humans.

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