

52



## DEPARTMENT OF HEALTH & HUMAN SERVICES

FDA/CFSAN/OFAS/DBGNR

### Memorandum

Date: July 14, 2005

From: Timothy P. Twaroski, Ph.D.  
Food and Drug Administration, Center for Food Safety and Applied  
Nutrition, Office of Food Additive Safety, Division of Biotechnology  
and GRAS Notice Review.

Subject: FAP 9M4682

To: Lane Highbarger, Ph.D.  
Consumer Safety Officer, Division of Biotechnology and GRAS  
Notice Review

#### Toxicology Memorandum

Re: FAP 9M4682; Ionizing radiation for the control of *Vibrio* and other foodborne pathogens in fresh or frozen molluscan shellfish.

Toxicology was asked to address a comment, jointly submitted by the Center for Food Safety and Public Citizen (Critical Mass Energy and Env't Program), regarding a journal article (Raul, et al., 2002) that implies that there may be a correlation between the consumption of 2-alkylcyclobutanones (ACBs) and the promotion of colon carcinogenesis.

ACBs are products that appear to be derived in very small amounts from irradiation of fatty acids present in many food products. However, the safety of irradiated food products has previously been reviewed by many scientific bodies, including the World Health Organization (WHO), the Joint Expert Committee on the Wholesomeness of Irradiated Food of the Food and Agriculture Organization (FAO), as well as the International Atomic Energy Agency (IAEA) and the US

Food and Drug Administration. These organizations all agree that irradiated food products are safe.

Promotion, as a stage in chemical carcinogenesis, has been shown to be induced by many different classes of chemicals, some of which are naturally occurring in the human body, including hormones. In the stage of promotion, a dose-response effect will exhibit a measurable threshold and maximal effect, but the process is reversible, if a sustained exposure to a promoter is not maintained (Cassereit & Doull's Toxicology, the basic science of poisons, 2001).

The study titled Food Borne Radiolytic Compounds (2-Alkylcyclobutanones) May Promote Experimental Colon Carcinogenesis, was designed to determine if ACBs, specifically 2-tetradecylcyclobutanone (2-tDCB) (formed from stearic acid) and 2-(tetradec-5'-enyl)-cyclobutanone (2-tDeCB) (formed from oleic acid), would promote the carcinogenic effects of azoxymethane (AOM), which is known to induce colon preneoplastic lesions, adenomas, and adenocarcinomas in rats. The paper stresses in the discussion that the main conclusion of this study is that ACBs may be promoters of colon tumors. A short commentary by Chinthalapally V. Rao, Ph.D., submitted with the comment, echoes a cautious sentiment about promotion by ACBs based on the study's data, but also points out several shortcomings in the study.

Assessing tumor promotion in this study is difficult because of the very limited number of animals (6 male Wistar rats per group), the testing of only one concentration of the ACBs (with no rationale for this specific exposure), the length of the study, and the absence of control groups not receiving AOM (the initiating agent) as well as a non-vehicle control for comparison. The lack of a non-vehicle control group is important because ethanol, the vehicle in this study, has been linked to tumor promotion in peer-reviewed studies (Chhabra et al., *In Vivo* 10(3):265-284; 1996). When assessing the safety of a chemical based on animal data, the exposure to the chemical must be taken into account. As the

authors pointed out in their discussion, the actual exposure of rats to 2-ACBs was on the order of milligrams per kilogram body weight. In contrast, human exposure would be much less, in the range of micrograms per kilogram body weight, which is a thousand-fold less than the rat exposure study.

Making any convincing conclusions is difficult because the data appears to be inconsistent. This is demonstrated - as the authors point out in the results section - by the fact that the data showed no significant difference in tumor incidence between treatment groups, also there is no apparent difference in the number of aberrant crypt foci (ACF) per centimeter for any of the treatment groups (table 1), and it appears that only the total number of aberrant crypts (AC) was elevated in the 6 month treatment group receiving 2-tDeCB. In addition, when you look at the number of AC per ACF there is limited evidence to support the conclusion that the ACB treated rats were statistical different than the control rats.

To expand on the definition of ACF, ACF are groupings of single or multiple crypts; which have altered luminal openings, thickened epithelia and are larger than adjacent normal crypts. ACF have been observed in animals which have been exposed to colon specific carcinogens, i.e. AOM. However, unprompted development of ACF in rats has been observed, and ACF may persist as hyperplastic lesions if they do not degenerate. Evidence supporting any direct correlation between ACF induction and tumor development is still being gathered, thus any relationship between the two is at best equivocal. To further complicate the scientific hypothesis, it has also been shown that some compounds which may decrease the occurrence of ACF may increase the development of colon tumors. (Mori, *et al.*; Mutation Research 566:191-208, 2004)

In a short commentary by Chinthalapally V. Rao, Ph.D., also submitted with this comment and later published with additional information and thoughts (Rao, *Nutrition and Cancer* 46(2):107-109; 2003), Dr. Rao points out some of these same study design flaws. Specifically, Dr. Rao discusses, the lack of animals per groups, as well as lack of proper control groups, and explanations regarding justification for a lack of correlation between data on ACF and tumor outcome. In addition, Dr. Rao pointed out that the AOM-induced F344 rat is generally accepted as the ideal model to mimic human colon cancer and that the Wistar rats, which were used in this study, are considered to be more sensitive to treatment with AOM.

In these same commentaries, FDA notes, that Dr. Rao indicates that there is “ample” preliminary evidence for the possible genotoxic effect of ACBs citing a study by Delincee on 2-dodecylcyclobutanone (formed from palmitic acid) (Delincee and Pool-Zobel, *Radiat. Phys. Chem.* 52:39-42; 1998). Later studies, have shown 2-dodecylcyclobutanone to be negative when tested for genotoxicity (Sommers, *J of Agricultural and Food Chemistry* 51:6367-6370; 2003) (Gadgil and Smith, *Journal of Food Science* 69:C713-C716; 2004) (Sommers and Schiestl, *Journal of Food Protection* 67:1293-1298; 2004). Moreover, it is important to note that Dr. Rao’s commentary states that fatty acids themselves (which are the precursors to ACBs) are influential in the pathogenesis of colon cancer. Interestingly, Raul and his coauthors cite a paper by Sakaguchi et al. (*Cancer Research* 44:1472-1477; 1984) which points out that a diet with linoleic acid (an unsaturated fatty acid) causes a higher incidence of colon tumors than a diet with stearic acid (a saturated fatty acid). This is even more remarkable given the fact that the ACB 2-tDeCB, which is derived from oleic acid (an unsaturated fatty acid), is the only ACB tested that possibly shows any increase in promotion of AOM-initiated colon tumors.