# Information about the potential toxicity of 2-alkylcyclobutanones, a group of substances exclusively formed upon irradiation of food containing fat.

In a recent study *'Toxicological study to assess the risk associated with the consumption of irradiated fat-containing food'* a French-German research collaborative group in frame of an EU Interreg programme investigated the potential toxicity of 2-alkylcyclobutanones. The researchers - Eric Marchioni, Dominique Burnouf, Henry Delincée, Andrea Hartwig, Michel Miesch, Francis Raul and Dalal Werner - summarize their results as follows:

## Introduction.

Food irradiation is considered as a highly effective processing technology to improve and maintain food safety. Indeed this process applied on food products dramatically reduces the populations of pathogens, which are annually responsible for millions of food-borne illnesses worldwide. The World Health Organization and many state agencies around the world have endorsed food irradiation as a major contributor to public health preservation. Irradiation of fat-containing food generates a family of molecules, namely 2-alkylcyclobutanones (2-ACB), that result from the radiation induced breakage of triglycerides. These components present the same number of carbons (n) as their fatty acids precursors, and an alkyl chain of (n-4) carbons, branched in ring position 2. Until now, these molecules have been found exclusively in irradiated fat-containing food, and are thus considered as unique markers for food irradiation.

## Aim and Results of the study.

This work has been undertaken in order to evaluate the toxicological properties, if any, of these 2-ACB. Our collaborative approach included the following experiments:

1) Synthesis of the major 2-ACB's found in food, namely the 2-tetradecyl-, 2-tetradecenyl-, 2-dodecyl, 2-decylcyclobutanones and some of their derivatives. The new synthesis process, eventually after additional purification, yielded highly pure compounds that were further used for toxicological studies.

2) Quantification in various types of irradiated foods of the different 2-ACB's present: this analysis clearly highlighted the linear relationship between the concentration of these generated compounds and the absorbed dose, even for food irradiated at very high doses. Moreover, it clearly showed that the pattern and yield of 2-alkylcyclobutanones depend on the precursor fatty acid profile of the irradiated food.

3) Toxicological studies revealed that the 2-ACB's have cytotoxic properties under certain experimental conditions, both in bacteria and cultures of human cells. This toxicity is strongly modulated by the length of the aliphatic side chain: the shorter the chain, the more acute the toxicity.
   Genotoxicity has been demonstrated by the induction of oxidative damage in DNA - determined by alkaline unwinding - upon treatment by 2-ACB's in human cell lines, but was not evident employing DNA Comet Assay. Although in bacteria a

clear cytotoxic effect was observed, no mutagenic activity has been revealed yet under our experimental conditions, using the Ames test.
Finally, using an experimental colon carcinogenesis model in rats, 2-ACB's, when tested at a high concentration, potentiate the effect of an inducing carcinogen on the long term. This was revealed by the increase of colonic preneoplastic lesions and the development of a higher number of colon tumours with larger size, as compared to animal controls, which were not exposed to 2-ACB's. This suggests that, in this model experiment, 2-ACB's, although they do not induce carcinogenesis *per se*, rather promote the colonic carcinogenic process. Finally, it was shown that small fractions of 2-ACB's had been stored in rat adipose tissues and excreted in faeces of the treated rats. This indicates that most of the 2-ACB's is metabolically transformed or stored in other organs.

## Conclusion.

The experiments demonstrate that pure compounds, known to be exclusively formed upon irradiation of fat-containing food, exhibit some toxic effects including promotion of colon carcinogenesis in rats. However, no mutagenicity could be observed using the Ames test. Whether these findings are relevant to the human exposure situation needs to be carefully considered. In our opinion further investigations, including confirmation of our results by other laboratories, will help to elucidate a possible risk associated with the consumption of irradiated fat-containing foods.

This work has been supported by the European Union through the Upper Rhine Interreg II Programme, and by Aérial (Strasbourg), Bundesforschungsanstalt für Ernährung (Karlsruhe), CNRS, Karlsruhe University, and Université Louis Pasteur (Strasbourg).