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Highbarger, Lane A

From: Kuznesof, Paul M
Sent: Monday, April 28, 2003 9:10 AM
To: Pauli, George H; Highbarger, Lane A; Lubin, Lisa; Harris, Rudolph
Subject: FW: Trans Fatty Acids - Irradiated ground beef

My comments on paper –see below

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-----Original Message-----

From: Paul M. Kuznesof [<mailto:vze2jv7f@verizon.net>]
Sent: Saturday, April 26, 2003 4:04 PM
To: Kuznesof, Paul M
Subject: Trans Fatty Acids - Irradiated ground beef

Re. M.S. Brito, A. L. C.H. Villavicencio, and J. Mancini-filho, "Effects of irradiation on *trans* fatty acids formation in ground beef," *Rad. Physics Chem*, 63, 337-340 (2002).

This is a strange paper!

(Note: samples irradiated "at 25C (room temperature)"!)

The title of section 2.4 is "Trans fatty acids analysis". The content of 2.4 is a description of a standard method for analyzing fatty acids in foods. The word *trans* is not used in the section. So there is no hint about how TFAs were identified and analyzed (which I doubt they were!).

The second paragraph in "Results and discussion" declares "The main *trans* fatty acid group in ground beef is 18:1." No reference is provided and no basis for this assertion is given. Certainly the data in Table 1 (fatty acid composition) for non-irradiated beef cannot be used to support the statement.

First sentence of the first paragraph on p. 339: "There is a decrease of fatty acid and an increase of *trans* fatty acid...." [presumably from irradiation, and presumably demonstrated by the data in Tables 1-3]. First, there is no basis for saying that there is a decrease in fatty acids, as all columns add up to 100%. Second, there's no basis for saying there's an increase in *trans* fatty acids because there are no data in Tables 1-3 on *trans* fatty acids (although Table 4 says the data in Table 4 are for TFAs).

The second paragraph on p. 339 declares: "The results showed an increase in *trans* fatty acids related to

the increase on irradiation dose.." and that irradiation induces TFA formation and refers to Table 4. The header of Table 4 explicitly notes that the data relate to TFAs. Only the good lord knows where these data come from. First, the data presumably show that TFAs are present in unirradiated beef at 4.5-5%. No reference cited. Then, at 1 kGy, the TFA level nearly doubles to about 8.0%. No further increase can be seen at doses of 2, 3, 4, and 5 kGy (8.0-8.5% TFAs). At 6 and 7 kGy the levels rise to 9.5% and at 8 kGy to 10-11%. Conclusion: the numbers, for what they are worth or whatever they mean, show no obvious dose/response.

The last paragraph on p. 339 addresses lipid oxidation oxidation. Its relevance to the TFA issue is hard to see.

Next-to-last para. of R and D: Reiterates that TFAs increase as a result of irradiation. And also notes that the data in Tables 1-3 show that linoleic acid (18:2) levels decrease due to lipid oxidation. Of course, there's no basis for such a conclusion.

At the bottom of Tables 2 and 3 there is a line for "Other" unsaturated fatty acids. No line in Table 1. It's interesting how at 8 kGy (at room temp irradiation) about 10-12% of total FAs is now unidentified unsaturated FAs, something the authors missed and might have been worth commenting on.

Finally, one observes that the standard deviations for all the numbers in the Tables raise suspicions about the data, in general. Compare the SDs for the three columns in each of the Tables. Within a Table, each column has the same SDs for a given fatty acid. Also, the similarity of the values of FA content (not the SDs) in Tables 1-3 for the C14-C18 saturated FAs is truly amazing!

BOTTOM LINE: Paper should never have been published. No evidence that trans fatty acids were ever identified in the study. No dose/response of the data purporting to show that levels of TFAs increase with dose increase. Numbers are suspicious. One also wonders about the meat being irradiated at 25C! Certainly not appropriate as a basis for drawing conclusions on increased health risk - unless such data could be extrapolated down to temperatures at which beef would actually be irradiated (something that was not addressed.)



MEMORANDUM

Date: April 28, 2003

From: Toxicology Group 2, Division of Food Contact Notification (DFCN) , HFS-275

To: Regulatory Group1, DBGNR, HFS-255
Attn: Lane A. Highbarger, Ph.D.

Subject: Requested comments on the COMET assay and cyclobutanone studies

Through: Chingju Sheu, Ph.D. *Chingju Sheu*
Group Leader, Toxicology Group 2, DFCN

Comments on the COMET assay

The COMET assay (CA) is an elegant and rapid assay that measures DNA breakage in *in vivo* and *in vitro* mammalian systems. Several refinements have been recently added to this assay to make it more specific to certain types of DNA damage like DNA-DNA and DNA-protein cross-linking. There are many important issues that have not been resolved with the COMET assay. I will list a few:

- The mechanism(s) of DNA damage has not been fully elucidated. Theoretically, alkaline labile sites are generated which result in DNA breakage. This is a good hypothesis but formal proof is lacking.
- Since DNA single-stranded breaks (ssb's) are not the major form of DNA damage for most chemical genotoxins, interpretation of the results is not a straightforward matter. Both DNA alkaline labile sites and ssb's are probably intermediates in the cellular repair process.
- The relevance of this assay is unclear since a relationship with either mutagenesis or cancer has not been elucidated. There are COMETS that do not have biological significance in terms of mutagenesis.
- Many confounding factors exist, specially with the use of the CA in human biomonitoring. Heavy exercising, diabetes, infection, sun light exposure, and smoking can cause increased COMET frequencies. Extensive variability can originate from small differences in pH during alkaline treatment or during isolation of cells from tissues because of spontaneous release of endonucleases that can breakdown the DNA.
- So far, the CA has not been validated and there is no standard protocol. Statistical issues need to be worked out since there are a number of ways to measure DNA damage, i.e., tail length, tail fluorescence, percentage of the DNA in the COMET head and in the tail, responder cells, non-responder cells, etc.

In conclusion, the CA has not yet reached the level of reliability and reproducibility that is needed to be considered a standard procedure for testing potential genotoxins. At present, the assay is valuable in basic research of cellular responses to DNA damage and repair in both *in vitro* and *in vivo* systems.

Cyclobutanones and DNA damage

With regard to the issue that cyclobutanones can cause significant DNA damage, I can say that the potential genotoxicity of 2-dodecylcyclobutanone (2-DCB) has not been determined in standard tests yet. The paper by Delincée and Pool-Zobel (*Radiat. Phys. Chem.* 52:39-42, 1998) on the induction of DNA damage by 2-DCB has serious deficiencies. Briefly, the genotoxicity of 2-DCB was measured by the COMET assay using rat and human colon cells. One of the major deficiencies with this paper is the lack of statistical analysis of the data. The standard errors of the mean (SEM) for each of the mean DNA damage responses were so large that no conclusion could be drawn regarding a dose-response effect. Furthermore, the differences between the human and rat DNA damage responses are probably not statistically significant, given the large SEM's. Another problem is that the doses used were 1000 to 2000 times larger than the actual human intake (a fact recognized by the authors). To my knowledge, no further studies on this matter have been reported by these authors.

My conclusion is that the data are preliminary and inconclusive and should not be used to state that 2-DCB can cause significant DNA damage.



Rene E. Sotomayor, Ph.D.
OFAS/DFCN
April 28, 2003