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## MEMORANDUM

**Date:** April 28, 2003

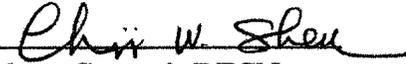
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**From:** Toxicology Group 2, Division of Food Contact Notification (DFCN), HFS-275

**To:** Regulatory Group 1, DBGNR, HFS-255  
Attn: Lane A. Highbarger, Ph.D.

**Subject:** Requested comments on the COMET assay and cyclobutanone studies

**Through:** Chingju Sheu, Ph.D.   
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.

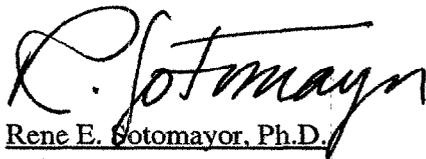
In conclusion, the CA has not yet reached the level of reliability and reproducibility that is needed to be considered a standard procedure for testing potential genotoxins. At present, the assay is valuable in basic research of cellular responses to DNA damage and repair in both *in vitro* and *in vivo*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

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### 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

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