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Date May 21, 2012
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Mutagenic Potential of Dihydroxyacetone (DHA): Comments on Petersen et al. (2004)

In a publication from 2004, Petersen et al. reported the induction of DNA damage in cultured HaCat keratinocytes incubated with high concentrations of the skin tanning agent Dihydroxyacetone (DHA) [1]. These results might suggest a certain mutagenic activity of DHA, however, several aspects and results from other studies have to be taken into account when assessing the data of this publication and the mutagenic/genotoxic potential of DHA:

1. The test system used by Petersen et al. was an *in vitro* comet assay detecting genotoxic (not mutagenic) effects in single cells. This *in vitro* test system is not part of standard test batteries for mutagenicity testing and interpretation of results with regard to mutagenicity and carcinogenicity are often difficult and still under discussion. In addition, the design of the experiment from Petersen (e.g. the cell line used) and the analysis of cells are very uncommon making a sound scientific interpretation of the data very difficult.

2. The DNA damage observed by Petersen et al. (2004) occurred only at very high DHA concentrations (50 – 100 mM), which exceeds the maximum test material concentration recommended by international accepted guidelines for *in vitro* mutagenicity testing (10 mM). Moreover, the effects on DNA occurred only at cytotoxic concentrations (> 10 mM) and, therefore, represent most probably typical *in vitro* artifacts.

3. The mutagenic potential of DHA was explicitly investigated by others using a battery of *in vitro* and *in vivo* mutagenicity assays: While DHA was mutagenic in the bacterial mutagenicity assay in bacteria (Ames assay) at high concentrations [2], it was non-mutagenic in the *in vitro* mammalian cell gene mutation assay (HPRT test) up to concentrations reaching the solubility limits of the compound [3]. In addition, DHA was non-clastogenic in an *in vitro*

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mammalian chromosome aberration assay [4]. Finally, DHA was not genotoxic/mutagenic in an *in vivo* micronucleus assay in mice treated intraperitoneally with high doses of DHA [5]. All these *in vitro* and in vivo tests, which are part of standard testing batteries for the evaluation of the mutagenic potential of substances, were performed according to international accepted guidelines and, with the exception of the *in vivo* study, under GLP regulations.

4. Dihydroxyacetone was not carcinogenic in Swiss-Webster mice after topical application of aqueous solutions of 5% and 40% DHA. [6]

5. Topical application of DHA to hairless mice irradiated with UV doses significantly delayed the time to appearance of tumors [7].

Based on all this data and applying the weight of evidence approach it can reasonably be concluded, that DHA is evaluated as not mutagenic and not carcinogenic. This was confirmed in 2010 by an evaluation of the Scientific Committee on Consumer Safety of the European Commission, which evaluated DHA as safe in cosmetic formulations at its present use [8]. Therefore, the data observed by Petersen et al. (2004) are considered of no biological relevance for the situation in humans.

Darmstadt, May 21, 2012

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