Food and Chemical Toxicology 84 (2015) 161–168

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Review

# "Aspartame: A review of genotoxicity data"

David Kirkland [a, *], David Gatehouse [b]

[a] Kirkland Consulting, P O Box 79, Tadcaster, LS24 0AS, United Kingdom
[b] Old Barn, Cherry Orchard Lane, Wyddial, Near Buntingford, Herts SG9 0EN, United Kingdom

ARTICLE INFO

*Article history:*
Received 12 June 2015
Received in revised form
19 August 2015
Accepted 23 August 2015
Available online 28 August 2015

*Keywords:*
Aspartame
Genotoxicity *in vitro*
Genotoxicity *in vivo*

ABSTRACT

Aspartame is a methyl ester of a dipeptide of aspartic acid and phenylalanine. It is 200× sweeter than sucrose and is approved for use in food products in more than 90 countries around the world. Aspartame has been evaluated for genotoxic effects in microbial, cell culture and animal models, and has been subjected to a number of carcinogenicity studies. The *in vitro* and *in vivo* genotoxicity data available on aspartame are considered sufficient for a thorough evaluation. There is no evidence of induction of gene mutations in a series of bacterial mutation tests. There is some evidence of induction of chromosomal damage *in vitro*, but this may be an indirect consequence of cytotoxicity. The weight of evidence from *in vivo* bone marrow micronucleus, chromosomal aberration and Comet assays is that aspartame is not genotoxic in somatic cells *in vivo*. The results of germ cell assays are difficult to evaluate considering limited data available and deviations from standard protocols. The available data therefore support the conclusions of the European Food Safety Authority (EFSA) that aspartame is non-genotoxic.



**Contents**



*Abbreviations:* CA, chromosomal aberrations; CBPI, cytokinesis-block proliferation index; EFSA, European Food Safety Authority; GLP, Good Laboratory Practice; ICH, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use; MI, mitotic index; MN, micronucleus or micronuclei; MNNCE, micronucleated normochromatic erythrocytes; MNPCE, micronucleated polychromatic erythrocytes; NCE, normochromatic erythrocyte; NNG, net nuclear grains; NTP, National Toxicology Program; OECD, Organisation for Economic Co-operation and Development; PCE, polychromatic erythrocyte; RI, replication index; UDS, unscheduled DNA synthesis.

* Corresponding author.
*E-mail address:* dkirkland@genetoxconsulting.co.uk (D. Kirkland).

http://dx.doi.org/10.1016/j.fct.2015.08.021

## 1. Introduction

Aspartame is a methyl ester of a dipeptide composed of aspartic acid and phenylalanine. The chemical structure is shown in Fig. 1. There are two forms of aspartame, an α and a β form, but only the α form is sweet. It is approximately 200 times sweeter than sucrose. It is approved for use in a wide range of food products in more than 90 countries around the world and is estimated to have been added to over 6000 different products (Butchko and Stargel, 2001).

Aspartame has been extensively evaluated for genotoxic effects in microbial, cell culture and animal models. It has also been subject to a number of carcinogenicity studies. The carcinogenicity of aspartame was reviewed in detail by Magnuson et al. (2007), and more briefly by Yilmaz and Uçar (2014), and has been the subject of a number of regulatory opinions (SCF, 2002; EFSA, 2006, 2009a, 2009b, 2011a, 2013). However, the genotoxicity of aspartame was only briefly reviewed by both Magnuson et al. (2007) and by Yilmaz and Uçar (2014). The latter authors concluded that aspartame was a moderate genotoxic agent, whereas Magnuson et al. (2007) concluded that extensive *in vitro* and *in vivo* studies provide ample evidence that aspartame is not genotoxic. The purpose of this manuscript is therefore to describe in more detail and to critically review all the available genotoxicity data on aspartame.

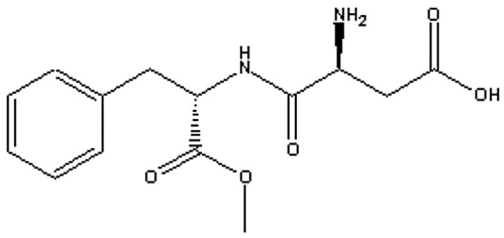


**Fig. 1.** Structure of aspartame.

## 2. *In vitro* studies

A number of *in vitro* genotoxicity studies, including bacterial, chromosomal aberration, micronucleus and DNA repair tests, have been performed on aspartame. The studies and their findings are summarised in Table 1. Brief additional comments on the significance of the findings are given below.

### 2.1. Bacterial mutagenicity

The available Ames tests with aspartame are summarised in Table 1. It can be concluded from the combined results of these four studies that aspartame is not a mutagen for bacterial cells. It is noted that none of the studies fully comply with current regulatory guidelines in that a bacterial test strain for the detection of

**Table 1**
Summary of *in vitro* genotoxicity studies with aspartame.

| Test system | Target cells | Concs. tested | Test conditions | Results | Reference |
|---|---|---|---|---|---|
| Bacterial mutation (Ames) assays | *S. typhimurium* TA1535, TA1537, TA98, TA1538, TA100 | 10–5000 μg/plate | Pre-incubation method − & + S9 in 2 independent expts. | No toxicity; no significant increases in revertant counts | Molinary (1978) |
| | *S. typhimurium* TA98, TA100 | 50–2000 μg/plate | Plate incorporation − & + S9 in 2 independent expts., though data from only 1 expt. reported | No significant increases in TA98 revertant counts; small increases (max. 1.4-fold, not dose-related) in TA100 revertants not considered biologically significant | Rencuzogullari et al. (2004) |
| | *S. typhimurium* TA1535, TA1537, TA98, TA97, TA100 | 100–10,000 μg/plate | Pre-incubation method −S9 and with 10% hamster liver S9; 30% hamster liver S9; 10% rat liver S9 and 30% rat liver S9; 2 independent expts. | Negative results in TA100, TA1535, TA1537 and TA98 under all test conditions. At 10,000 μg/plate in TA97 (30% rat liver S9) a 1.4-fold increase in revertants was judged equivocal. | NTP (2005) |
| | *S. typhimurium* TA97a, TA100 | 10–1000 μg/plate | Plate incorporation method − & + S9, single experiment | No toxicity; no increases in revertant counts | Bandyopadhyay et al. (2008) |
| Chromosomal aberrations (CA) | Human peripheral blood lymphocytes from 2 male & 2 female donors | 500–2000 μg/mL | 24 or 48 h treatment in the absence of S9 inducing up to 34 or 56% mitotic inhibition; 400 cells/conc. scored for CA | Statistically significant increases in CA frequency at all 3 test concentrations after both treatment periods | Rencuzogullari et al. (2004) |
| Micronuclei (MN) | Human peripheral blood lymphocytes from 2 male & 2 female donors | 500–2000 μg/mL | 24 or 48 h treatment in the absence of S9; cytochalasin B present for the final 24 h; 8000 binucleate cells/conc. scored for MN | A small (1.9–2.2-fold) but statistically significant increase in MN frequency seen at 2000 μg/mL for both treatment periods. | Rencuzogullari et al. (2004) |
| Unscheduled DNA synthesis (UDS) | Cultured hepatocytes from male Sprague–Dawley rats. | 5 and 10 mM | 20 h treatment; 150 cells/conc. scored for autoradiographic grains | Net nuclear grain counts all <zero, so no induction of UDS | Jeffrey and Williams (2000) |
| Mitotic recombination | *Aspergillus nidulans* diploid strain UT448/UT196 | 100–12,000 μg/mL | 6 days treatment; 30–69 mitotic haploids per link interval at each conc. | Increases in the occurrence of haploid mitotic segregants and mitotic recombination at concs. of 800 and 1000 μg/mL | Gebara et al. (2003) |