# French Food Safety Agency

## ASSESSMENT REPORT

## Opinion
## on a possible link between the exposition to aspartame and the incidence of brain tumours in humans

## 1 – Context of the request

The French Food Safety Agency (Agence française de sécurité sanitaire des aliments; Afssa) was requested, on October 16, 2000, by the "Direction générale de la concurrence, de la consommation et de la répression des fraudes" to assess the possible link between the exposition to aspartame and the incidence of brain tumours in humans.

Aspartame is authorised in France as a sweetener to be used in table-top products and in a number of foods (French Official Journal, October 2, 1997).

In articles published in the media and on the Internet (ATIC, 1998), consumption of aspartame has been implicated in the occurrence of a number of adverse effects in humans. According to these publications, this sweetener could have toxic effects on the central nervous system and, in particular, it might be implicated in an increase in the incidence of brain cancers in the United States.

This report has been produced by the Afssa Specialist Experts Committee on Additives, Flavourings and processing aids based on the available scientific literature. The aim of this report would be to reassess: (i) the toxicity of aspartame on the central nervous system in laboratory animals and in humans, (ii) the exposure of French consumers to this sweetener and, (iii) the public health risk related to the consumption of aspartame.

At the European community level, the Scientific Committee on Food (SCF) is currently conducting an assessment of the scientific data on the effects attributed to aspartame. This assessment is being conducted by the British Food Standards Agency (FSA). Accordingly, the Afssa and the FSA are working in partnership, with the Afssa's contribution on the evaluation of the neurological effects of aspartame, which will be included in the FSA report.

## 2 - Introduction

Aspartame was discovered in 1965 by a chemist working for the American company Searle and an initial marketing authorisation (MA) was granted in the United States by the Food and Drug Administration (FDA) in 1974. This MA was suspended a few months later following an appeal against the authorisation on the grounds that the toxic and carcinogenic effects on the brain of this compound and its metabolites had not been properly evaluated during the experimental studies. Following a reassessment of the studies on experimental animals and an examination of new data (including a study of carcinogenicity in the rat), the FDA granted this product a new MA in 1981 (FDA, FR 1981) for use in solid food. This authorisation was extended to soft drinks in 1983 (FDA, FR 1983) and for its use as a general sweetener in 1996. The safety of aspartame has been assessed and recognised by a number of other national and international organisations including the FAO/WHO Committee of Experts on Food Additives (JECFA) and, at EU level, by the Scientific Committee on Food. It was authorised by Directive 94/35/EC of the European Parliament and of the Council on sweeteners for use in foodstuffs (adopted on 30 June 1994) and its use is permitted in more than 90 countries. In France, aspartame has been permitted since 1988. The Acceptable Daily Intake (ADI) of aspartame for humans was fixed at 40 mg/kg body weight/day by the JECFA (1980).

In 1996, an article by J.W. Olney suggesting a link between an increased incidence of brain tumours in the United States and the marketing of aspartame relaunched the debate on the risks to human health posed by its consumption. The debate has been covered by the media, notably on the Internet where several thousand websites are devoted to the effects of aspartame. These contain allegations claiming that this additive is responsible for a large number of adverse effects (more than fifty), some of which are very serious, such as: multiple sclerosis, lupus erythematosus, Gulf War syndrome, brain tumours, epileptic seizures, complications of diabetes, etc. At the same time, the health authorities in a number of countries have reacted by informing the public on the studies available or underway and on the data based on scientific evidence.

Following a recap of the physical and chemical properties of aspartame, this report will review, firstly, the available toxicological and epidemiological data on the effects of this additive on the nervous system (cancer and seizures) and secondly, the estimates of the consumption of this sweetener by the general population and by specific populations such as children and diabetic adolescents. For these specific populations, there may be a potential risk arising from heavy consumption, low body mass or finally, a special metabolic susceptibility.

## 3 – Identity, physical and chemical properties and stability of aspartame

The E number of aspartame in France and Europe is E 951. It was first marketed by NutraSweet AG and more recently by Ajinomoto and Holland Sweetener Company. This sweetener is incorporated into a number of foodstuffs (drinks, desserts, sweets, etc.) and in table sweeteners, under the name Canderel, Pouss-suc and into some 600 medicines; this report does not consider the possible intake (very limited compared with dietary intake) of aspartame from this later source. Its sweetening power is 180 to 200 times greater than that of saccharose.

Aspartame is a dipeptide methyl ester of L-aspartyl-L-phenylalanine. It is a white, odourless, crystalline powder. Its molecular weight is 294.3 Daltons and its rotatory power $[\alpha]_D^{22} = 2.3°$ in 1M HCl.
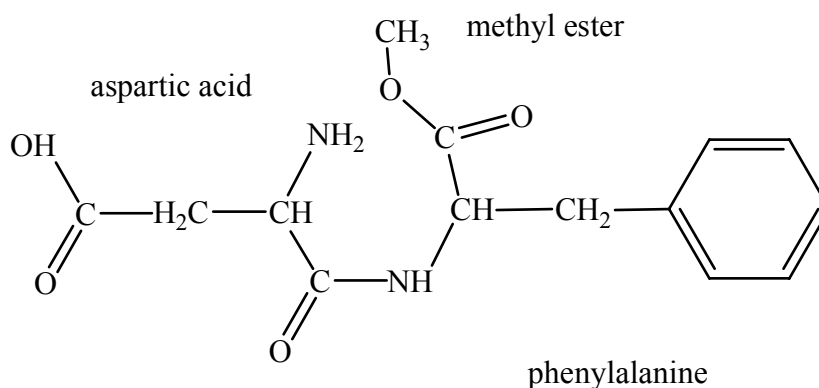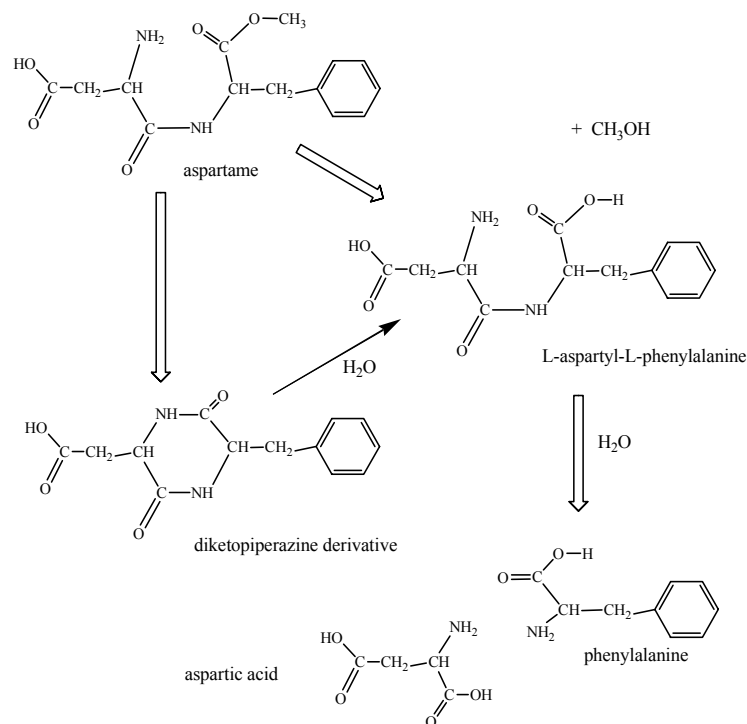


Its main impurity (approximately 2%) is diketopiperazine, a degradation product of aspartame which has no sweetening properties.

The solubility of aspartame in water is dependent on pH and temperature, the maximum solubility is reached at pH 2.2 (20 mg/ml at 25°C) and the minimum solubility at pH 5.2 (pHi) is 13.5 mg/ml at 25°C.

The stability of aspartame is dependent on time, temperature, pH and water activity (Dziezak, 1986; Bell *et al.*, 1991; Tsoubeli *et al.*, 1991; Homler, 1984; Graves *et al.*, 1987; Huang *et al.*, 1987; Neiderauer, 1998).

Aspartame is very stable in the dry state: at 105°C a loss of approximately 5% (formation of diketopiperazine) is observed after 100 hours of treatment. At 120°C, a 50% loss is obtained after 80 hours of treatment.

In solution, when stored at temperatures ranging from 30 to 80°C, aspartame is progressively degraded into diketopiperazine (Pattanaargson *et al.*, 2000). It is therefore not usable in foods heated at higher temperature (cooking, sterilisation, etc.). At room temperature its stability is good at pH values of between 3.4 and 5 and it is maximum at pH 4.3. At pH below 3.4 the dipeptide is hydrolysed and at a pH greater than 5, cyclisation occurs with the formation of diketopiperazine. In both cases, this transformation results in the loss of sweetness.

aspartame

+ $CH_3OH$

$H_2O$

L-aspartyl-L-phenylalanine

$H_2O$

diketopiperazine derivative

phenylalanine

aspartic acid

In foods with a low or moderate water content (water activities between 0.34 and 0.66), the maximum stability is observed at pH 5.0.

Aspartame has good stability in deep frozen products.

# 4 – Metabolism and toxicology

Both in laboratory animals and in humans, aspartame is metabolised in the gastro-intestinal tractus into methanol, aspartic acid and phenylalanine. On a weight basis, metabolism of aspartame generates approximately 50% phenylalanine, 40% aspartic acid and 10% methanol. Metabolism occurs in the intestinal lumen and in the enterocytes (Karim *et al.*, 1996; Stegink *et al.*, 1996). It has been reported that a small proportion of the aspartame (10-12% of the intake) might be absorbed without metabolisation but this result requires confirmation (Creppy *et al.*, 1998).

## 4.1 – Long term toxicity and carcinogenicity of aspartame

### Experimental data

Aspartame is not genotoxic in a reverse mutation test on *S. typhimurium*, in two chromosome aberration tests *in vivo* on somatic cells and in Rodent dominant lethal test on germ cells (JECFA, 1980). Recently, two studies have confirmed the absence of clastogenic potential (Durnev *et al.*, 1995; Mukhopadhyay *et al.*, 2000) of the compound.

In a carcinogenicity study on CD-1 mice (FDA, FR 1981), aspartame administered in feed at doses of 1, 2 and 4 g/kg bw./day for 110 weeks, showed no carcinogenic potential.

Three carcinogenicity studies were conducted in Sprague Dawley and Wistar rats.

- In the first study (1973), post-weaning Sprague Dawley rats were fed doses of aspartame corresponding to 1, 2, 4, 6/8 g/kg bw./day for 104 weeks (6/8 i.e. dose of 6 was increased during the study to 8 g/kg bw./day).

- In the second study (1974), male and female Sprague Dawley rats, from a two-generation study, were exposed during gestation, lactation and after weaning for 104 weeks, to doses of 0, 2 and 4 g/kg bw./day in their food.

The results of these two studies have been widely discussed by the scientific community and the regulatory authorities (FDA). In the first study, the incidence of brain tumours in the treated animals was higher than in the control animals but without any dose-response relationship. In contrast, in the second study the incidence of tumours in the treated rats was lower than in the control group. For these reasons, a third study was conducted under conditions of Good Laboratory Practice in order to ensure the reliability of the experimental data.

- In this third study (Ishii, 1981), groups of male and female Wistar rats were given doses of aspartame of 0, 1, 2, 4 g/kg bw./day for 104 weeks. Under these conditions, aspartame did not cause any increase in the incidence of brain tumours.

Taking into account all the studies that have been conducted, the frequency of spontaneous tumours in laboratory rats, the types of tumours observed and the absence of a dose-response relationship, it was concluded that aspartame had no carcinogenic potential on the brain in experimental animals (FDA FR, 1981-1984; Koestner, 1984; Cornell et al., 1984; Flamm, 1997).

The Acceptable Daily Intake (ADI) of aspartame for humans was established at 40 mg/kg bw. (JECFA, 1980) based on a no-observed effect level (NOEL) of 4 g/kg of bw./day, which was the highest dose retained during the carcinogenicity studies in rats, divided by a safety factor of 100.

**Epidemiological data**

In 1996, Olney et al. published an article on a possible link between the increase in the frequency of brain tumours in humans and the consumption of aspartame in the United States. Based on the data from the National Cancer Institute (10% of the population) from 1975-1992, the authors concluded that there was a significant increase in the frequency of brain tumours in the mid-1980s, that is to say the period following aspartame came onto the market.

The conclusions of this epidemiological study have been criticised by a number of scientists who questioned the methodology, the use of the data and their interpretation (Levy et al., 1996; Linet et al., 1999; Ross, 1998; Seife, 1999; Smith et al., 1998). One of the major criticism is that the authors only took into account the frequency of brain tumours during a selected period (1975-1992). When all the epidemiological data are used (1973-1992) a different conclusion is reached, as the frequency of brain cancers began to increase in 1973 and stabilised from the mid-1980s (Levy et al., 1996). Furthermore, Olney et al. did not provide any quantitative or qualitative relationship between the exposure of the population to aspartame and the observed frequency of brain tumours. Finally, an increase in the incidence of the tumours can have many causes including, among others, improvements in diagnostic methods (Modan et al., 1992).

More recently, Gurney et *al*., (1997) published the results of a case-control study on the relationship between the consumption of aspartame and the frequency of brain tumours. The study covered 56 patients affected by tumours in childhood and 94 controls. According to these authors, no relationship could be established between the consumption of aspartame and the frequency of brain tumours.

In France, data on the incidence of and mortality from brain cancers were supplied by the FRANCIM network (F. Ménégoz, 2001). These cancers include meninges tumours and tumours of the brain itself (see graphs in the annex). Between 1980 and 1997, the incidence (number of new cases appearing each year) of cerebral tumours was relatively stable in men and showed a slight increase in women. The trend towards an increase in mortality from cancer of the brain and other parts of the nervous system is a longstanding one, as it first appeared in 1950 and continues to the present day, for both sexes. However, during the last decade, mortality in men stabilised and the increase in mortality from brain cancer in women was less pronounced than during the preceding period.

Within this context, it is worth noting that a case-control study is currently underway in France (CEREPHY - Laboratoire Santé, Travail, Environnement, Université de Bordeaux II) which concerns the relationship between brain cancers and exposure to plant protection products and also lifestyles (including the consumption of sweeteners). The results of this study could provide additional information and act as a guide for future epidemiological research. Similarly, the results to be published from the epidemiological study conducted by the National Cancer Institute between 1994 and 1998 will require careful analysis. This study has assessed the possible relationship between various factors (mobile phones, dietary factors such as sweeteners, etc…) and the incidence of brain tumours in adults.

In France, the epidemiological data from the cancer registers do not enable a definitive indication to be given on a possible aspartame-brain tumour relationship, but they do show that, at the present time, the sale of this food additive in France is not being accompanied by an increase in the frequency of brain tumours or increased mortality from this disease in the general population.

**4.2 – Relationship between aspartame and epileptic seizures in animals and humans**

Among the possible adverse effects of aspartame, researchers have paid particular attention to seizures. Several studies have suggested a relationship between the consumption of large amounts of aspartame and the triggering of epileptic seizures. In an old study (1972), on new-born monkeys (2-3 animals per group) treated with doses of aspartame of 1, 3 and 4 g/kg bw./day for 52 weeks, epileptic seizures were recorded at the highest doses, after 218 days of treatment. Thereafter, sporadic convulsions were observed during handling of the animals. These symptoms were identical with those observed in young monkeys treated with phenylalanine. In contrast, in a similar study also conducted on young monkeys, no effect was observed at doses of aspartame of 2 and 2.7 g/kg bw./day. The different results observed in the two studies could be explained by differences in the exposure conditions, the food and the state of health of the animals (JECFA, 1980).

Walton *et al*. (1993) reported, in a study conducted on 13 patients suffering from depression, that the administration of 30 mg/kg bw./day of aspartame for 7 days caused severe side effects in these patients which led the authors to conclude that the use of this sweetener in depressive patients should be avoided. The same author (Walton, 1986) reported a case of

epileptic seizure and serious behavioural problems in a woman being treated with anti-depressants who ingested large quantities of tea containing aspartame. Wurtman (1985) indicated that the administration of aspartame, due to an increase in phenylalanine absorption in the brain, could affect the synthesis of catecholamines or serotonin and cause seizures. He based his findings on three examples of heavy consumers of "diet" drinks and on experimental studies on animals demonstrating that the consumption of aspartame reduced the threshold of sensitivity to chemically induced seizures (Maher *et al.*, 1987 ; Guiso *et al.*, 1988; Pinto *et al.*, 1988). Finally Camfield et *al.* (1992) demonstrated that aspartame could increase the duration of certain types of epileptic seizure in children.

The ATIC on the Internet reported a large amount of evidence from people who have identified aspartame as the cause of their health problems and in particular of seizures. These statements should be taken into account but with the reservation that they have not been examined according to any academic standard. They may, however, in certain cases, reflect the hypersensitivity of certain individuals to aspartame or its metabolites. Effects on seizures have been reported with phenylalanine, aspartic acid and methanol but these were under specific conditions (high doses, individual sensitivity, types of seizures, etc.) which are not representative of the general population and of current use of this sweetener in food (Anderson *et al.*, 1996).

This causal relationship between aspartame and epileptic seizures has been refuted by a large number of scientists who base their opinions on numerous experimental studies conducted on laboratory animals or on clinical or tolerance studies in humans (Anderson *et al.,* 1996; Gaull, 1985; Rowan et *al.,* 1995; Shaywitz et *al.,* 1994; Tollefson *et al.*, 1992; 1993; Dailey et *al.*, 1991; Zhi et *al.*, 1989; Sze, 1989; Tilson et *al.*, 1989). The Epilepsy Institute in the USA has also concluded that aspartame is not the cause of epileptic seizures (Congressional Record, June 20, 1986).

In the United States various consumer complaints about aspartame have been collected by the Special Nutritionals Adverse Event Monitoring System (SN/AEMS). The sources of these reports were the FDA, federal and local health agencies, consumers and health professionals. Of 2621 side effects reported, concerning 3451 products, some ten cases concerned preparations concerning aspartame (mixtures also containing vitamins, amino acids and various nutritional supplements). The effects reported included seizures, death, nervous and cardiac symptoms, oedema and fever. Still in the United States, the Center for Disease Control assessed 517 complaints about aspartame (1983). The symptoms reported were headaches, mood changes, insomnia, abdominal pain, nausea, convulsions, etc… These symptoms are observed frequently in the general population. Although it might be possible that certain individuals are particularly sensitive to aspartame, these data, which relate to a large number of people, have not enabled any relationship to be demonstrated between the consumption of aspartame and the occurrence of convulsive seizures.

**4.3 – Toxicity of aspartame metabolites on the nervous system**

In both laboratory animals and in humans, aspartame is metabolised in the intestinal tract into methanol, aspartic acid and phenylalanine.

**Methanol**

Methanol accounts for approximately 10% of aspartame in terms of weight. It is metabolised into formaldehyde, formic acid and $CO_2$. One litre of "diet" drink containing aspartame produces approximately 48 mg of methanol whereas a litre of fruit or vegetable juice contains approximately 200 to 280 mg of methanol. This indicates that the quantities of methanol provided by aspartame as a food additive are lower than those provided by certain natural foods (Maher, 1986). In humans, ingestion of methanol at a dose of 200-500 mg/kg body weight is required to induce an accumulation of formate in the blood and toxic effects on the vision and the central nervous system. These doses are more than 100 times greater than the maximum dose of methanol provided by aspartame (FDA, FR 1984). These data put into perspective the potential toxic effects of the metabolites of methanol (formaldehyde and formic acid) following exposure to aspartame.

Trocho et *al.,* (1998) demonstrated that aspartame, radio-labelled on the methanol, induced in the liver stable DNA and proteins adducts . According to these authors, the accumulation of these adducts after repeated administration of aspartame could pose problems of toxicity and carcinogenicity in the long term. Besides the fact that aspartame at high doses has never induced liver cancer in rats, Trocho's studies did not identify the radioactivity found in the proteins and DNA. Consequently, the formation of adducts of formaldehyde on the proteins and nucleic acids from aspartame, *in vivo*, remains to be proved (Tephly, 1999).

**Aspartic acid**

The transformation of aspartame into aspartic acid do not pose any neurotoxicity effects , notably due to the characteristics of the transport system for this amino acid across the blood-brain barrier which prevents its accumulation in the brain by promoting its transport toward the blood (Maher, 1986). Aspartic acid is mostly eliminated through the lungs in the form of $CO_2$. In humans, plasma levels of aspartic acid do not increase significantly following oral administration of aspartame at a dose of 34 mg/kg bw. (Stegink, 1984).

**Phenylalanine**

Some of the phenylalanine formed in the intestine following ingestion of aspartame is excreted in the form of $CO_2$, most of it is incorporated into the pool of amino acids and contributes to protein synthesis.

The following table shows the plasma concentrations of phenylalaninee measured after oral administration of aspartame at different doses in adult men (Trefz *et al*., 1996; Stegink *et al*., 1996):

| Aspartame (mg/kg) | Phenylalanine (µmol/l) | |
| --- | --- | --- |
| | Baseline concentrations | Plasma Peak Concentrations |
| 34 | 50- 60 | 110 – 120 |
| 50 | - | 160 – 170 |
| 100 | - | 200 |
| 150 | - | 350 |
| 200 | - | 500 |
| Children with PKU | 1200 - 6000 | |

PKU = phenylketonuria

Even for doses far higher than normal exposure doses, baseline blood concentrations of phenylalanine likely to cause side effects in children suffering from phenylketonuria are not reached. Furthermore, the administration of repeated doses of aspartame does not lead to an accumulation of phenylalanine in the blood.

According to Maher *et al.* (1986, 1987), the ability of phenylalanine to reach the brain differs depending on whether it originates from the consumption of aspartame or from the ingestion of dietary proteins. There may be a competition results at the blood-brain barrier between phenylalanine and other amino acids. This means that the consumption of aspartame other than at mealtimes would be liable to increase selectively the level of phenylalanine in the brain. This phenomenon could lead to a special risk for people with a phenylalanine-hydroxylase deficiency and individuals with neurological disorders such as epilepsy. This latter point is supported by Wurtman (1985) who indicated that the administration of aspartame, by increasing the absorption of phenylalanine in the brain, could affect the synthesis of catecholamines or serotonin and cause convulsions.

According to the FDA (FDA, FR 1984) and several authors (reviewed by Stegink *et al.*, 1996; Stegink *et al.*, 1996(a); Trefz *et al.*, 1996), the doses of phenylalanine liable to produce toxic effects on the brain are much greater than those which could be produced by the consumption of aspartame tablets or in "diet" drinks. Furthermore, the effects of aspartame on the synthesis of neurotransmitters are still working hypotheses.

## 4.4 – Toxicity of dicetopiperazine in terms of effects on the nervous system

The toxicity of diketopiperazine, a degradation product of aspartame, has been studied in laboratory animals. This substance is neither genotoxic nor carcinogenic in rats and mice. The Acceptable Daily Intake of diketopiperazine for humans has been established at 7.5 mg/kg body weight (JECFA, 1980) based on the no observed effect level of 750 mg/kg bw./day obtained in a long term toxicity study on rats, divided by a safety factor of 100.

**4.5 - Conclusion**

The hypothesis of Olney that suggested that the exposition to aspartame could be a possible cause of the increase in the incidence of brain tumours in humans has not, to date, been confirmed by experimental data on animals or by the epidemiological data on humans.

A review of published data demonstrates the lack of evidence for establishing a causal link between aspartame and epileptic seizure or abnormal electroencephalograms in humans.

In comparison with current foodstuffs, aspartame is a minor source of phenylalanine, aspartic acid and methanol. These compounds cannot therefore be the source of toxic effects in consumer under normal conditions of use of this sweetener.

The safety of aspartame has been assessed and acknowledged by a number of national and international authorities, including the FDA, the JECFA and the SCF. Aspartame was authorised by the Directive 94/35/CE of the European Parliament and of the Council on sweeteners for use in foodstuffs (adopted on 30 June 1994) and its use is approved in more than 90 countries. The ADI for humans has been 40 mg per kg of body weight since 1980.

# 5 – Estimate of the consumption of aspartame in France

The dietary consumption of aspartame has been estimated in consumer studies in general population conducted on the adult and child. Additionally, consumption estimates have been produced for the adult and child diabetic populations who, according to the SCF, are the most exposed.

Dietary consumption was estimated from surveys, the aspartame content of food being taken as either the statutory theoretical and maximum level, or measured through analysis of artificially sweetened food products.

Based on purchasing information on vector foods for aspartame and using the maximum permitted values, a mean consumption was obtained of 0.4 mg/kg bw./d which represents 1% of the ADI.

*In adults*, based on aspartame consumption studies in the general population and using values of aspartame measured in food, mean consumption was between 0.05 and 0.4 mg/kg bw./d and maximum values between 1 and 2.75 mg/kg bw./d. This represents at worst 7% of the ADI for 10% of the population (Bär *et al.*, 1992; OCA/CREDOC, 1994; Hinson *et al.*, 1992).

*In children and adolescents*, based on aspartame consumption studies in the general population and using values of aspartame measured in food, maximum values were between 0.13 and 2.8 mg/kg bw./d (Hinson *et al.*, 1992 ; Leclercq *et al.*, 1999). These are identical with the maximum values found for the general population and represent less than 7% of the ADI.

*In diabetic or on a calorie-controlled diet adults*, Bär *et al.* (1992), using values of aspartame measured in food, demonstrated that adults on calorie-controlled diets were heavier consumers of aspartame than diabetics: mean consumption was 0.39 mg/kg bw./d which

corresponds to the highest mean value for adults in the general population. Maximum values were between 1.28 et 10.1 mg/kg bw./d. For 3.5% to 10% of this population, the values could, depending on the study, be as much as 3 to 25% of the ADI (Bär *et al*., 1992; MAFF, 1995; Hinson *et al*., 1992).

*Diabetic children* are considered an at-risk group for the consumption of sweeteners, firstly because the diabetic diet partially excludes simple carbohydrates and secondly due to their low body mass. Two studies have been conducted, one in Sweden and the other in France, using the maximum permitted values for aspartame in food.

- The Swedish study (Ilbäck *et al*., 2000) carried out a simulation of extreme consumption, by taking the consumption of the 10 subjects who were the most consumers of sweeteners (out of 320 subjects who took part in the survey). Mean weight was 20 kg and the estimated consumption of aspartame was 46 mg/kg bw./d or 114% of the ADI. These data should be used with care as the maximum values concerned only ten individuals and the aspartame contents used are the maximum levels permitted in food. Moreover, the dietary survey method used (frequency questionnaire) is imprecise and was not validated.

- The French study (Garnier-Sagne *et al*., 2001), was conducted on a population of 227 young diabetics (112 girls and 115 boys), members of the association "Aide aux Jeunes Diabétiques" [Young Diabetics Support], distributed over 65 out of the 95 French "Départements" and aged from 2 to 20. The method used was the 5-day diary. More than 84% of the diabetic children consumed sweeteners. The mean consumption of aspartame was estimated at 1.9 mg/kg bw./d (less than 5% of the ADI) and maximum intake was 15.6 mg/kg bw./d (40% of the ADI). The results were identical for boys and girls. This study also indicated that sugar-free drinks and table-top sweeteners were the forms in which aspartame was the most commonly consumed, contributing to 56% and 16%, respectively, of the estimated consumption.

In conclusion, estimates of aspartame consumption have not demonstrated any exceeding of the ADI, in particular in France, even in diabetic children who are the heaviest consumers of aspartame. It is worth noting that in view of the increasing levels of obesity in children, another population consuming large amounts of sweeteners, it would be interesting to have consumer surveys for them.

# 6 – Summary and conclusion

Aspartame is stable at room temperature. The major degradation product at higher temperatures is diketopiperazine. These two compounds have been satisfactorily assessed in a large number of studies in humans and an ADI has been established for each of them: 40 mg/kg bw. for aspartame and 7.5 mg/kg bw. for diketopiperazine.

The metabolites of aspartame in humans are compounds found in normal foods and which are also produced by the endogenous cellular metabolism. Aspartame is a minor source of phenylalanine, aspartic acid and methanol compared with the standard dietary intake of these substances. They cannot therefore, be the source of the toxic neurological effects attributed to aspartame.

Genetic toxicity assays have demonstrated that aspartame and diketopiperazine are not genotoxic.
None of the carcinogenicity tests that have been conducted on rodents indicated a relationship between treatment with aspartame and the appearance of brain tumours.

The epidemiological study by Olney *et al.* which suggested a link between the placing on the market of aspartame and a possible increase in the frequency of brain cancers in humans did not provide any scientific evidence to justify or demonstrate a basis for this suggestion; to date it has not been confirmed.

Analysis of the scientific literature has demonstrated a lack of evidence based on the current state of knowledge which would enable a causal link to be established between the consumption of aspartame and the occurrence of epileptic seizures or anomalies on an electro-encephalogram. (It has been verified in France that no anti-convulsive medication contains high quantities of aspartame).

The consumption of aspartame in humans, even in particularly exposed populations such as diabetic children, does not exceed the Acceptable Daily Intake, notably in France.

**In conclusion, Afssa considers that the current state of scientific knowledge does not enable a relationship to be established between the exposition to the aspartame and brain tumours in humans or animals.**

# 7 – Bibliography

## General scientific works

The clinical evaluation of a food additive. Assessment of Aspartame. Tschanz C, Butchko HH, Stargel WW, Kotsonis FN, Edts, 1996, CRC Press, Boca Raton, New York, London, Tokyo.

Aspartame. Physiology and Biochemistry. Steginck LD, Filer LJ. Edts, 1984, Marcel Dekker, Inc, New York and Basel.

## Publications and documents

Anderson GM, Novotny EJ, Shaywitz BA. Evaluation of seizures. In: The clinical evaluation of a food additive. Assessment of Aspartame. Tschanz C, Butchko HH, Stargel WW, Kotsonis FN, Edts, 1996, pp 205-216, CRC Press, Boca Raton, New York, London, Tokyo.

ATIC - Aspartame Toxicity Information Center, 1998,  http://www.holisticmed.com/aspartame/

Bär A, Biermann Ch. Intake of intense sweeteners in Germany. *Zeitschrift für Ernährungswissenschaft*, 1992, 31, 25-39.

Bell L.N., Labuza T. Aspartame degradation kinetics as affected by pH in intermediate and low moisture systems. *J. Food Sci.*, 1991, 56, 17.

Bradley D. Aspartame link "not proven", *Chem. Britain*, 1997, 33, 7.

Camfield PR, Camfield CS, Dooley JM, Gordon K, Jollymore S, Weaver DF. Aspartame exacerbates EEG spike wave discharge in children with generalised absence epilepsy : a double blind controlled study. *Neurology*, 1992, 42(5), 1000-1003.

Cornell RG, Wolfe RA, Sanders PG. Aspartame and brain tumours: statistical issues. In : Aspartame: Physiology and Biochemistry, 1984, 459-479.

Creppy EE, Baudrimont I, Betbeder AM. How aspartame prevents the toxicity of ochratoxin A. *J. Toxicol. Sci.*, 1998, 23(suppl 2), 165-172.

Dailey JW, Lasley SM, Burger RL, Bettendorf AF, Mishra PK, Jobe PC. Amino acids, monoamines and audiogenic seizures in genetically epilepsy-prone rats: effects of aspartame. *Epilepsy Res.*, 1991, 8(2), 122-133.

Durnev AD, Oreshchenko AV, Kulakova AV, Beresten NF, Seredenin SB. Clastogenic activity of dietary sugar substitutes. *Vopr. Med. Khim.* 1995, 41(4), 31-3.

Dziezak J.D. Sweeteners and product development, *Food Technol*. January 1986, 119-121.

Food and Drug Administration, Federal Register, 1981, 1983, 1984.

Flamm WG. Increasing brain tumour rats: is there a link to aspartame? *J. Neurpathol. Exp. Neurol.*, 1997, 56(1), 105-106.

Gaull GE. Aspartame and seizures. *Lancet*. 1985, 2 (8469-70),1431.

Garnier-Sagne, Leblanc JC, Verger Ph. Calculation of the intake of three intense sweeteners in young insulin-dependent diabetics. *Food  Chem. Toxicol*., 2001, 39(7), 745-749.

Graves D.J., Luo S. Decomposition of aspartame caused by heat in the acidified and dried state, *J. Agric. Food Chem*., 1987, 35, 439-442.

Guiso G, Caccia S, Vezzani A, Stasi MA, Salmona M, Romano M, Garattini S. Effect of aspartame on seizures in various models of experimental epilepsy. *Toxicol. Appl. Pharmacol*, 1988, 96(3), 485-493.
Gurney JG, Pogoda JM, Holly EA, Hecht SS, Preston-Martin S. Aspartame consumption in relation to childhood brain tumour risk: results from a case-control study. *J. Nat. Cancer Inst*. 1997, 89 (14), 1072-1074.

Hinson AL, Nicol WM. Monitoring sweetener consumption in Great Britain. *Food Addit. Contam*., 1992, 9(6), 669-681.

Homler, B.E. Properties and stability of aspartame. *Food Technol*., July 1984, 50.

Huang T.C., Soliman A.A., Rosen R.T., Ho C.T. Studies of the Maillard browning reaction between aspartame and glucose, *Food Chem*. , 1987, 24, 187-196.

Ilbäck NG, Alzin M, Jahrl S, Enghardt Barbieri H, Busk L. 2000. Sweetener intake and exposure. Study among Swedish diabetics. Report 2. National Food Administration, Sweden.

Ishii H, Incidence of brain tumours in rats fed aspartame, *Toxicol. Lett.*, 1981, 7, 433-437.

JECFA, Toxicological evaluation of certain food additives : aspartame, WHO food additive series No 15, Report series No 653, 1980.

JO - Journal Officiel de la République Française, 08/11/1997. Arrêté du 2 octobre 1997 relatif aux additifs pouvant être employés dans la fabrication des denrées destinées à l'alimentation humaine.

Karim A, Burns T. Metabolism and pharmacokinetics of radiolabeled aspartame in normal subjects. In: The clinical evaluation of a food additive. Assessment of Aspartame. Tschanz C, Butchko HH, Stargel WW, Kotsonis FN, Edts, 1996, pp 57-66, CRC Press, Boca Raton, New York, London, Tokyo.

Koestner A, Aspartame and brain tumours: pathology issues. In Aspartame: Physiology and Biochemistry, 1984, 447-457.

Koestner A, Increasing brain tumour rates: is there is a link to aspartame ?, *J. Neurpathol. Exp. Neurol.*, 1997, 56, 107-109.

Leclercq C, Berardi D, Rita Sorbillo M Lambe J. Intake of saccharin, aspartame, acesulfame K and cyclamate in Italian teen-agers: present levels and projections. *Food Addit. Contam*., 1999, 16, 99-109.

Legler JM, Ries LA, Smith MA, Warren JL, Heineman EF, Kaplan RS, Linet MS. Brain and other central nervous system cancers: recent trends in incidence and mortality. *J. Nat. Cancer Inst.,* 1999, 91(6), 1382-1390.

Levy PS, Hedeker D. Statistical and epidemiological treatment of the SEER incidence data. *J. Neurpathol. Exp. Neurol.*, 1996, 55(12), 1280.

Linet MS, Ries LA, Smith MA, Tarone RE, Devesa SS. Cancer surveillance series: recent trends in childhood cancer incidence and mortality in the United States. *J. Natl. Cancer. Inst*., 1999, 91(16), 1382-1390.

MAFF, 1995, Survey of the intake of sweeteners by diabetics. Food Safety Directorate Food Surveillance Information Sheet, p 76.

Maher TJ, Neurotoxicology of food additives. *Neurotoxicology*, 1986, 7(2), 183-196.

Maher TJ, Wurtman RJ. Possible neurologic effects of aspartame, a widely used food additive. *Environ.. Health Perspect.*, 1987, 75, 53-57.

Modan B, Wagener DK, Feldman JJ, Rosenberg HM, Feinleib M. Increased mortality from brain tumours: a combined outcome of diagnostic technology and change of attitude towards the elderly. *Am. J. Epidemiol.*, 1992, 135, 1349-1357.

Mukhopadhyay M, Mukherjee A, Chakrabarti J. In vivo cytogenetic studies on blends of aspartame and acesulfame-K. *Food Chem. Toxicol*., 2000, 38(1), 75-77.

Neiderauer T. Manufacture, properties and applications of sweeteners in foods, *Fluessiges-Obst.*, 1998, 65, 131-140.

OCA/CREDOC. 1994. La consommation d'édulcorants de synthèse en France, évolution de 1989 à 1992. Etat d'avancement de la base de données de l'Observatoire au 30 décembre 1994.

Olney JW, Farber NB, Spitznagel E, Robins LN.  Increasing brain tumour rates: is there is a link to aspartame ?, *J. Neurpathol. Exp. Neurol.*, 1996, 55(11), 1115-1123.

Pattanaargson S, Sanchavanakit C. Aspartame degradation study using electrospray ionization mass spectrometry. *Rapid Commun. Mass Spectrom.,* 2000, 14(11), 987-93.

Pinto JM, Maher TJ. Administration of aspartame potentiates pentenetrazole- and fluorothyl-induced seizures in mice. *Neuropharmacology*, 1988, 27(1), 51-55.

Roberts HJ. Aspartame and brain cancer, *The Lancet*, 1997 , 349, 362.

Ross JA. Brain tumours and artificial sweeteners? A lesson on not getting soured on epidemiology. *Med. Pediatr. Oncol.*, 1998, 30 (1), 7-8.

Rowan AJ, Shaywitz BA, Tuchman L, French JA, Luciano D, Sullivan CM. Aspartame and seizure susceptibility: results of a clinical study in reportedly sensitive individuals. *Epilepsia*, 1995, 36(3), 270-275.

SEER Cancer Statistics Reviews, 1973-1996. National Cancer Institute. Bethesda MD, 1999. URL:http://seer.cancer.gov/Publications/CSR1973_1996

Seife C. Increasing brain tumour rates: is there a link to deficit spending, *J. Neurpathol. Exp. Neurol.*, 1999, 58(4), 404-405.

Shaywitz BA, Anderson GM, Novotny EJ, Ebersole JS, Sullivan CM, Gillespie SM. Aspartame has no effect on seizures or epileptiform discharges in epileptic children. *Ann. Neurol.*, 1994, 35(1), 98-103.

Smith MA, Freidlin B, Ries LA, Simon R. Trends in reported incidence of primary malignant brain tumours in children in the United States. *J. Natl. Cancer Inst.*, 1998, 90 (17), 1269-77.

Stegink, LD. Aspartame metabolism in humans : acute dosing studies. In  Aspartame : Physiology and biochemistry, Stegink L, Filer L eds., 1984, 509-553, New-York, Marcel Dekker.

Stegink LD. The aspartame story : a model for the clinical testing of a food additive. *Am. J. Clin. Nutr.*, 1987, 46, 204-215.

Stegink LD, Filer LJ. Effects of aspartame ingestion on plasma aspartate, phenylalanine and methanol concentrations in normal adults. In: The clinical evaluation of  a food additive. Assessment of Aspartame. Tschanz C, Butchko HH, Stargel WW, Kotsonis FN, Edts, 1996, pp 67-86, CRC Press, Boca Raton, New York, London, Tokyo.

Stegink LD, Filer LJ. Effects of aspartame ingestion on plasma aspartate, phenylalanine and methanol concentrations in potentially sensitive populations. In: The clinical evaluation of  a food additive. Assessment of Aspartame. Tschanz C, Butchko HH, Stargel WW, Kotsonis FN, Edts, 1996(a), pp 87-113, CRC Press, Boca Raton, New York, London, Tokyo.

Sze PY. Pharmacological effects of phenylalanine on seizure susceptibility : an overview. *Neurochem. Res.*, 1989, 14(2), 103-111.

Tephly TR. Comments on the purported generation of formaldehyde and adduct formation from the sweetener aspartame. *Life Sci.,* 1999, 65(13), 157-160.

 Tilson HA, Thai L, Zhao D, Sobotka TJ, Hong JS. Oral administration of aspartame is not proconvulsant in rats. *Neurotoxicology*, 1989, 10(2), 229-238.

Tollefson L, Barnard RJ. An analysis of FDA passive surveillance reports of seizures associated with consumption of aspartame, *J. Am. Diet. Assoc.*, 1992 , 92(5), 598-601.

Tollefson L. Multiple chemical sensitivity : controlled scientific studies as proof of causation. *Regul. Toxicol. Pharmacol.*, 1993 , 18 (1), 32-43.

Trefz FK, Bickel H. Tolerance in PKU heterozygotes. In:  The clinical evaluation of  a food additive. Assessment of Aspartame. Tschanz C, Butchko HH, Stargel WW, Kotsonis FN, Edts, 1996, pp 149-160, CRC Press, Boca Raton, New York, London, Tokyo.

Trocho C, Pardo R, Rafecas I, Virgili J, Remesar X, Fernandez-Lopez JA, Alemany M. Formaldehyde derived from dietary aspartame binds to tissue components in vivo. *Life Sci.*, 1998, 63(5) 337-349.

Tsoubeli M.N. , Labuza T. Accelerated kinetic study of aspartame degradation in the neutral pH range. *J. Food Sci.*, 1991, 6, 1671.

Vetsch W. Aspartame: technical considerations and predicted use, *Food Chem.*, 1985, 16, 245-258.

Walton RG, Hudak R, Green-Waite RJ. Adverse reactions to aspartame: double blind challenge in patients from a vulnerable population. *Biol. Psychiatry*, 1993, 34 (1-2), 13-17.

Walton RG. Seizure and mania after high intake of aspartame. *Psychosomatics*, 1986, 27(3) 218, 220.
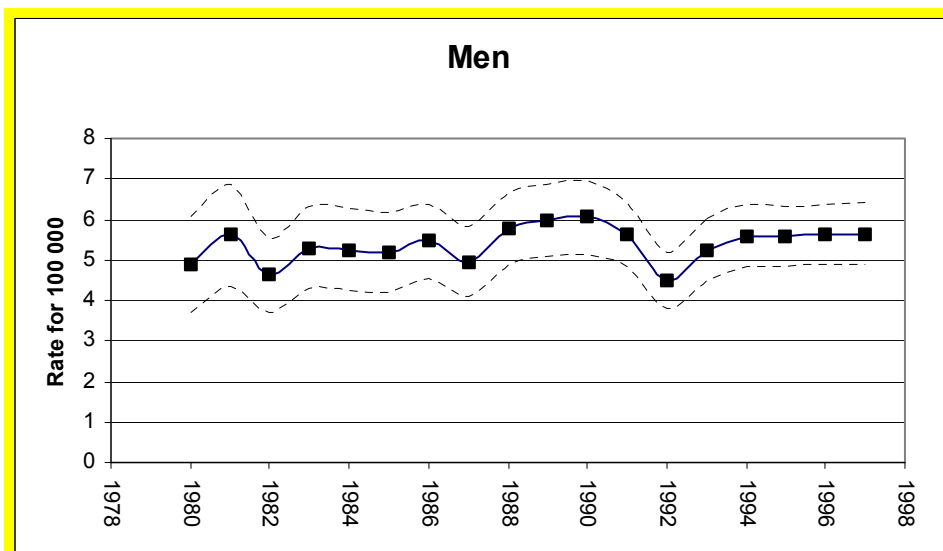
Wurtman RJ. Aspartame: possible effect on seizure susceptibility. *The Lancet*, 1985, 9(2), 1060.

Zhi JQ, Levy G. Aspartame and phenylalanine do not enhance theophylline-induced seizures in rats. *Res. Commun. Chem. Pathol. Pharmacol*. 1989, 66 (1),171-174.
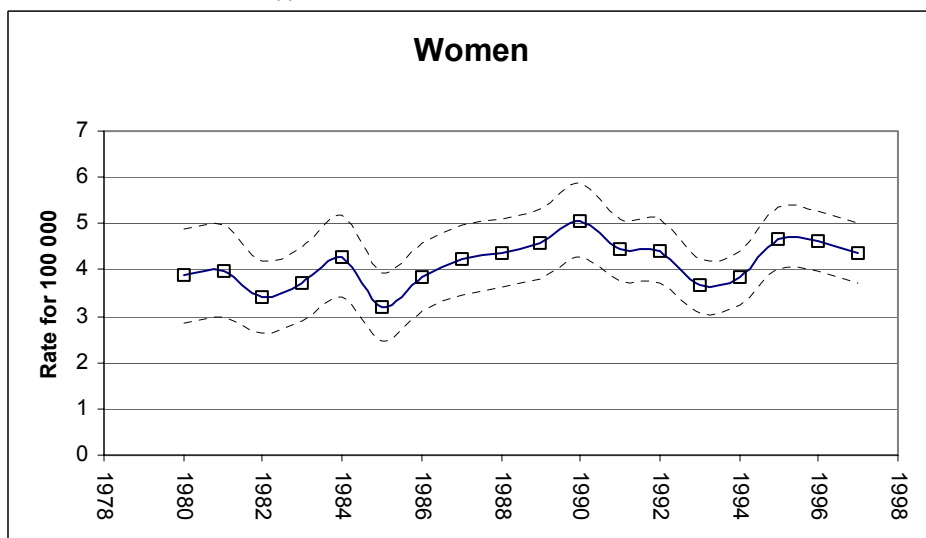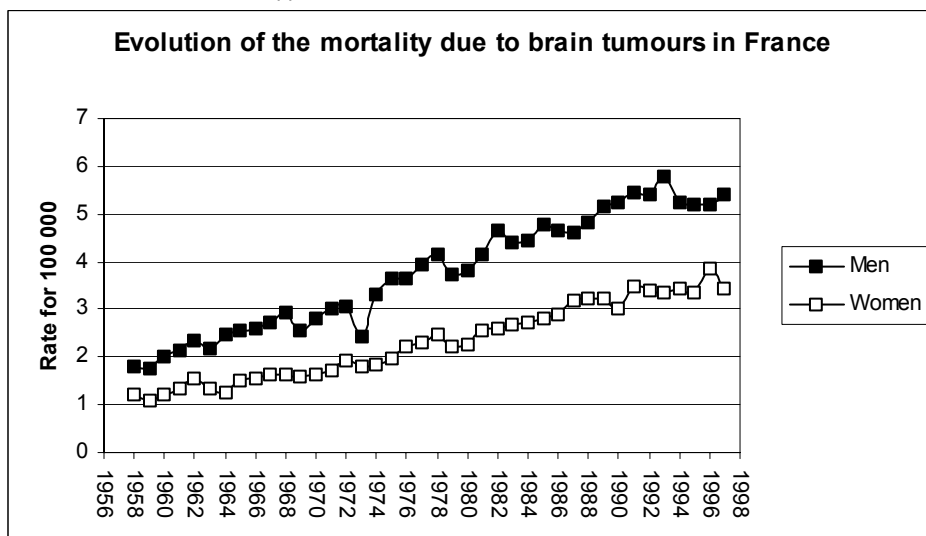
# ANNEXES

## Incidence(*) of Brain tumours (Meninges and encephalon)
## In the French registers($)

### Men



Rate for 100 000

The doted lines indicate the upper and lower limits of the confidence interval at 95%

### Women



Rate for 100 000

The doted lines indicate the upper and lower limits of the confidence interval at 95%

### Evolution of the mortality due to brain tumours in France



Rate for 100 000

Men
Women

**(*) The incidence is the number of new cases reported each year**
**($) Source of data: FRANCIM network (2001)**