



**EUROPEAN COMMISSION**  
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL  
Directorate B - Scientific Health Opinions  
**Unit B3 - Management of scientific committees II**

**Scientific Committee on Food**

**SCF/CS/ADD/EDUL/194 final**  
**13 March 2000**

## **Opinion**

### **Re-evaluation of acesulfame K**

**with reference to the previous SCF opinion of 1991**

(Expressed on 9 March 2000)

---

Rue de la Loi 200, B-1049 Bruxelles/Wetstraat 200, B-1049 Brussel - Belgium - Office: BE232 - 6/37.

Telephone: direct line (+32-2) 295.81.10 / 296.48.70, exchange 299.11.11. Fax: (+32-2) 299.48.91

Telex: COMEU B 21877. Telegraphic address: COMEUR Brussels.

[http://www.europa.eu.int/comm/dg24/health/sc/scf/index\\_en.html](http://www.europa.eu.int/comm/dg24/health/sc/scf/index_en.html)

## Opinion

### Re-evaluation of acesulfame K with reference to the previous SCF opinion of 1991 (Expressed on 9 March 2000)

#### Terms of Reference

To re-evaluate the safety and the Acceptable Daily Intake (ADI) of acesulfame K as a sweetener, in light of additional information received.

#### Background

Acesulfame K is a high intensity sweetener, with a sweetness approximately 200 times that of sucrose. It is used in a wide range of low or reduced calorie food products and beverages.

Acesulfame K was considered by the Committee for the first time during its comprehensive review of sweeteners in 1985 (1). The Committee established an ADI of 0-9 mg/kg, based on the no-observed-adverse-effect level (NOAEL) in a 2-year study in the dog, which was at that time regarded as the most sensitive species. Following a request by the petitioner to increase the ADI to 0-15 mg/kg, the review on the safety of acesulfame K was updated by the Committee in 1991 (2). In this evaluation the Committee found no new scientific basis for increasing the ADI. At that time, no toxicokinetic studies on possible species differences in systemic exposure were submitted by the applicant.

Acesulfame K has also been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), for the first time in 1983 (3) and subsequently re-evaluated in 1991 (4). At the first evaluation JECFA allocated an ADI of 0-9 mg/kg based on toxicological data in dogs. At the subsequent re-evaluation the ADI was raised to 0-15 mg/kg. The basis for this change in opinion was that acesulfame K is not metabolised in any tested species and that the 2-year study in rats represented a greater portion of life (compared to 2 years in dogs), and should thus be the basis for the ADI.

Since this Committee's last review, a further submission has been received from the same petitioner (5), containing new experimental data, information about by-product formation under experimental changes to the production process, proposed changes to the purity specifications to exclude the presence of by-products during normal production, and a further request to increase the ADI. In addition, a recently published study has reported a dose-dependent increase in chromosome aberrations in mice (6), raising doubts about the non-genotoxicity of acesulfame K. The Center for Science in the Public Interest (CSPI) in the USA has also put forward objections to the approval and use of acesulfame K (7), expressing concerns about the potential for carcinogenicity of acesulfame K and questioning the adequacy of the carcinogenicity bioassays conducted in the past. In this re-evaluation, all the above information has been considered alongside the earlier data.

## Review and re-evaluation of data

### Production procedures and purity specifications

The Committee was informed that the applicant now uses different starting materials from those originally employed and the applicant has requested adoption of the 1996 JECFA specifications on acesulfame K. These specifications conform to those of directive 95/31/EC, July 5th 1995, OJEC L178/28-7-95, with the exception of the following aspects. A new limit is included for the level of organic impurities (UV active compounds). This is to take account of the possible formation of 5-chloro-acesulfame as an impurity in the production procedure, should it deviate from normal. In the EC Directive limits are also set for arsenic, selenium and lead. Such specific limits do not exist in the JECFA specifications.

### Metabolism studies

Single oral doses of acesulfame K given to dogs and rats were rapidly absorbed and excreted mainly in the urine as unchanged compound (9,10). The excretion kinetics of acesulfame K in the rat is biphasic with an estimated half-life in the rapid phase of approximately 4 hrs (11,12). When single doses of acesulfame K were given orally to dogs and pigs, maximum blood levels were reached at 1-2 hrs after dosing (9,10). A single oral dose of 30 mg acesulfame K given to human volunteers was rapidly and almost completely absorbed (13). Maximum blood concentration was reached after 1-1.5 h and thereafter elimination occurred rapidly with a plasma half-life of 2-2.5 h. Only the parent compound could be identified in serum and urine, indicating that no significant degradation of acesulfame K had occurred.

New toxicokinetic studies in rats and dogs have been performed since the Committee's 1991 re-evaluation of acesulfame K (14,15). Acesulfame K was administered to animals of both sexes at two dose levels, via the diet in the rat and via the single, daily feed, i.e. as a bolus dose, in the dog. After 2 weeks of treatment the toxicokinetic profiles were determined during 24 hrs. There were no major sex differences in either species. The results are shown in Table 1. Only steady state and not peak plasma concentration ( $C_{max}$ ) values, could be obtained for rats since administration was by continuous feeding. The increases in  $AUC_{(24h)}$  in both species and in  $C_{max}$  in the dog were virtually proportional to dose.

**Table 1: Comparative toxicokinetics of acesulfame K in rats and dogs**

Species	$AUC_{(24h)}$ ( $\mu\text{g}\cdot\text{h}\cdot\text{ml}^{-1}$ )		Plasma concentration ( $\mu\text{g}\cdot\text{ml}^{-1}$ )	
	Rat	Dog	Rat St. state	Dog $C_{max}$
Lower dose <sup>1</sup>	848-934	2149-3819	16-71	180-311
Higher dose <sup>2</sup>	1521-1671	3065-5722	30-119	273-491

1 Rat c.840 mg/kg bw/day via diet; dog 900 mg/kg bw/day as bolus dose

2 Rat c.1325 mg/kg bw/day via diet; dog 1500 mg/kg bw/day as bolus dose

### Mutagenicity studies

A number of studies on acesulfame K indicate that the compound is not mutagenic. Negative results have been obtained in several bacterial assays with *S. typhimurium* and *E. coli* (16) and in mammalian cells *in vitro* (17). It did not induce micronuclei in NMRI mice (18) or chromosomal aberrations in Chinese hamster cells (19). Also malignant cell transformation (17), unscheduled DNA synthesis (20) and *in vivo* DNA binding studies were all negative (21).

In contrast to these results, a paper published in 1997 (6) reported a dose-dependent increase in chromosome aberrations in bone marrow cells of mice treated with acesulfame K. However, there are concerns about the experimental conditions and the adequacy of this study. Moreover, the positive results could not be repeated by another laboratory which conducted a follow-up study to current OECD guidelines, using the same chromosome aberration test in bone marrow cells and the same strain of Swiss Albino mice (22). The results showed that acesulfame K did not induce chromosome mutations. It is also noted that the same group that reported positive results in 1997 at doses of acesulfame K of 60mg/kg bw and above (6), failed to show any increase in chromosome aberrations in a subsequent study using the same assay system as before, with a mixture of aspartame and acesulfame K (150mg/kg bw) (27).

### Chronic toxicity and carcinogenicity

Three long-term/carcinogenicity studies have been performed on acesulfame K, one in the mouse (23) and two in the rat (24,25). These studies have previously been evaluated on several occasions, both by this Committee (1,2) and by JECFA (3,4) and no concerns were raised about carcinogenicity. However, the CSPI has raised concerns about the adequacy of the carcinogenicity studies in terms of the study protocols, the chosen dose levels and inappropriate use of historical controls (7). Furthermore, the CSPI has stated that all the studies indicate a possible carcinogenic effect of acesulfame K which are difficult to evaluate due to the experimental conditions.

In the mouse carcinogenicity study, Swiss mice were fed with a diet containing 0, 0.3, 1.0 or 3.0% of acesulfame K for 80 weeks (23). A slightly decreased body weight was recorded, but it reached significance only at a few scattered points in time. There were no significant increases in mortality or tumour incidence due to the treatment.

The first rat carcinogenicity study that was performed is not adequate and cannot be used for the safety evaluation of acesulfame K (24). The second carcinogenicity study used the same doses as in the mouse study, i.e. diets containing 0, 0.3, 1.0 or 3.0% acesulfame K (25). A slightly decreased body weight in the 3% dose group was recorded, but the decrease was minor and reached significance only at a few scattered points in time. Haematology and clinical chemistry parameters showed scattered changes, but no clear or dose-dependent pattern, indicating that they were of no toxicological significance. There were no significant increases in mortality or tumour incidence.

## Discussion

### Carcinogenicity and mutagenicity aspects

The Committee considered that although the carcinogenicity studies are old they could still be used in the safety evaluation of acesulfame K. Moreover, the Committee does not agree with the interpretation of the CSPI that there is an indication of possible carcinogenicity from these studies. The one aberrant, positive mutagenicity finding in mouse bone marrow cells could not be replicated and all other mutagenicity findings were negative. No other new data has appeared indicating potential harmful effects. Thus there is no reason to require any additional studies of chronic toxicity/carcinogenicity or mutagenicity.

### Toxicokinetic aspects

At the Committee's last evaluation, the dog was regarded as potentially the most sensitive and therefore the most appropriate species to use for setting the ADI. Since species-specific kinetic data were not available at that time, there was no scientific justification for using the rat instead of the dog to set the ADI. The newly submitted toxicokinetic data were examined to see if they show which species may be the most appropriate for setting the ADI.

Acesulfame K is rapidly absorbed and subsequently excreted in the urine at a comparable rate in all investigated species, with an estimated half-life of approximately 2-4 h. However, the new and previously available toxicokinetic data in humans (13), rats (9), pigs (10) and dogs (9,15) show that plasma peak concentrations ( $C_{\max}$ ) after a single bolus administration may differ between the species by up to an order of magnitude. A species comparison shows that each administered mg/kg b.w. of the compound results in maximum plasma concentrations of approximately 700 ng/ml in humans, 75 ng/ml in rats, 132 ng/ml in pigs and 272 or 656 ng/ml in two separate dog studies. Thus, based on these results it is likely that the absorption-elimination pattern and plasma peak concentrations are comparable in humans and dogs, whereas in rats the obtained plasma-peak concentration was several-fold lower.

Accordingly, the systemic exposure ( $AUC_{(24\text{ h})}$ ) to acesulfame K was shown to be 3- to 4-fold higher in dogs than in rats after administration of similar doses (see Table 1) (14,15). The difference in mode of administration (continuous via the diet in the rat and as a bolus in the single, daily feed in the dog) would be expected to affect plasma  $C_{\max}$  values, and may also account for the increase in  $AUC_{(24\text{ h})}$  values in the dog. Given the rapid absorption and excretion in both species, it is unlikely that any small differences in these aspects would account for the large differences in  $AUC_{(24\text{ h})}$ . It is noted that increases in  $C_{\max}$  and  $AUC_{(24\text{ h})}$  in both the rat and the dog were virtually proportional to the given dose, suggesting that absorption and excretion pathways remain unsaturated over the dose range tested.

Since no effects of toxicological significance were observed either at the top dose used in the 2-year dog study (900 mg/kg bw/day (27)) or at the top dose used in the 2-year rat study (1500 mg/kg bw/day (25)), no conclusion may be drawn about whether one species may be more sensitive to acesulfame K than the other. However, systemic exposure can be assumed to have been higher in the dog at 900 mg/kg bw/day (around 3000  $\mu\text{g}\cdot\text{h}\cdot\text{ml}^{-1}$ ) than in the rat at 1500 mg/kg bw/day (around 1600  $\mu\text{g}\cdot\text{h}\cdot\text{ml}^{-1}$ ).

Although the absorption, plasma half-life and excretion patterns appear to be similar in all species tested including humans, the peak plasma concentrations following bolus administration differ, being around 10-fold lower in the rat than in the human or dog. Use of sweeteners in soft drinks gives intake patterns which are closer to bolus administration than dietary administration. Moreover, total systemic exposure ( $AUC_{24hr}$ ) is higher in the dog than the rat for comparable doses, though some of this difference might be due to the differing route of administration in those particular studies. Because of the greater kinetic similarity between dog and human than between rat and human, the Committee considers that the dog should remain the appropriate species on which to base the ADI, using a NOAEL of 900 mg/kg bw/day obtained in the 2-year dog study. The Committee has considered whether a reduced safety factor could be used, given the metabolic similarity between dog and human with respect to peak plasma concentrations following bolus administration. However, the kinetic data are somewhat limited and so the default safety factor of 100 is still considered appropriate. Should better comparative kinetic data become available, then reconsideration may be possible.

## **Conclusion**

The Committee has evaluated material complying with the JECFA specification. It endorses the 1996 specification, in particular the inclusion of a limit on organic impurities, because of the potential for formation of 5-chloro-acesulfame, for which there are no toxicological data.

After re-evaluation of the earlier and new information on mutagenicity and consideration of the comments of the CSPI in relation to the earlier information on carcinogenicity, the Committee reaffirms its previous conclusions that acesulfame K is without mutagenic or carcinogenic potential. Furthermore, acesulfame K does not induce any other effects of toxicological significance at dietary dose levels up to 3% in the rat (equivalent to 1500 mg/kg bw/day) or in the dog (equivalent to 900 mg/kg bw/day).

In considering the request to increase the ADI from 0-9 mg/kg bw to 0-15 mg/kg bw, the Committee took into account previously available and new toxicokinetic data in a variety of species including man.

On the grounds of limited evidence of toxicokinetic similarity between humans and dogs and the observation that, for the same total daily dose, the plasma peak concentration and  $AUC_{(24h)}$  for the dog are several-fold higher than that for the rat, the Committee considers, that the dog remains the appropriate species on which to base the ADI and reaffirms its previous ADI of 0-9 mg/kg bw.

## References

1. European Commission (1985). Food-science and techniques. Report of the Scientific Committee for Food (Sixteenth series). CEC, Luxembourg.
2. European Commission (1991). Minutes of the 79th meeting of the Scientific Committee for Food held on 20-21 June in Bilthoven. DG III, Brussels. Document III/3432/91-EN.
3. Joint FAO/WHO Expert Committee on Food Additives. Toxicological Evaluation of Certain Food Additives. WHO Food Additives Series 18:12-14, 1983. WHO, Geneva.
4. Joint FAO/WHO Expert Committee on Food Additives. Toxicological Evaluation of Certain Food Additives. WHO Food Additives Series 28:183-218. WHO, Geneva.
5. Dossier-Addendum to "Application for using Acesulfame K as a sweetener. Summary dossier – proposal for re-evaluation". SCF Dossier EC 196.01, submitted by Nutrinova-Hoechst, 1999.
6. Mukherjee A and Chakrabarti J (1997). *In vivo* cytogenetic study on mice exposed to Acesulfame K – a non-nutritive sweetener. Food and Chemical Toxicology 35:1177-1179.
7. CSPI (1996). Center for Science in the Public Interest. Letter to the National Institute of Environmental Health Science/National Toxicology Program (NIEHS/NTP) dated 29th May 1996 (in Jnr. 571.2120/3135-0001).
8. JECFA (1996). Acesulfame K. Joint FAO/WHO Expert Committee on Food Additives. Compendium of food additive specifications (46<sup>th</sup> session). FAO Food and Nutrition Paper 52 (Add. 4). FAO, Rome.
9. Kellner HM and Christ O. Re-absorption, distribution and elimination after administration of Acetosulfam-<sup>14</sup>C to rats and dogs. Unpublished report Dr. Kn/tr 5547, 1975. Submitted by Hoechst A.G.
10. Kellner HM and Christ O. Pharmacokinetic studies of Acetosulfam-<sup>14</sup>C after oral administration to pigs. Unpublished report, 1975. Submitted by Hoechst A.G.
11. Volz M. and H Eckert. Unpublished Research Report No. 01-L42-0352-81, 1981. Submitted by Hoechst A.G.
12. Volz M., H Eckert and HM Kellner. Investigation into the kinetics and biotransformation of the sweetener Acesulfame K following repeated high-dose administration. Unpublished Research Report No. 01-L42-0396-83, 1983. Submitted by Hoechst A.G.
13. Volz M. Unpublished Research Report No. 01-L42-0177-76, 1976. Submitted by Hoechst A.G.
14. Troschau G. Acesulfame K. Toxicokinetics in rats after repeated-dose (2-week) administration in the diet. Report Study No. 95.0200, 1995. Pharma Development, Corporate Toxicology, Hoechst A.G, Frankfurt, Germany.
15. Stammberger I. Sunett® (Acesulfame-K). 14-day feeding study in dogs- Tolerability and Toxicokinetics. Study Report No. 98.0628, 1998. Drug Innovation & Approval, Department of Toxicology/Pathology, Hoechst Marion Roussel, Hattersheim, Germany. Submitted by Hoechst A.G.
16. Jung R and Hollander W. Study of the mutagenic potential of strains of *Salmonella typhimurium* (Ames test) and *Echerichia coli*. Unpublished report No.86 0811, 1986. Submitted by Hoechst A.G.

17. Marquardt HWJ. Mutagenicity and oncogenicity of Acesulfame K. Studies with mammalian cells in vitro. Unpublished report of the Memorial Sloan-Kettering Cancer Centre, N.Y., 1978. Submitted by Hoechst A.G.
18. Baeder C and Horstmann G. Oral embryotoxicity study of Acesulfame K in rabbit, stock Hoe: HIMK (SPF Wiga). Unpublished report No. 317/77, 1977. Submitted by Hoechst A.G.
19. Mayer D, Weigand W and Kramer M. Acesulfame K. Cytogenetic study in the Chinese hamster. Unpublished report No. 575/78, 1978. Submitted by Hoechst A.G.
20. Myhr BC and Brusick DJ. Evaluation of Acesulfame K "CIVO-4" in the primary rat hepatocyte unscheduled DNA synthesis assay. Unpublished report of Litton Bionetics No. 21001, 1982. Submitted by Hoechst A.G.
21. Sagelsdorff P, Lutz WK and Schlatter CH. Studies on DNA binding. Unpublished report No. 40182A, 1981. Submitted by Hoechst A.G.
22. Völkner W. Chromosome aberration assay in bone marrow cells of the mouse with Acesulphame K. RCC-CCR Project No. 609900, 1998. RCC Cytotest Cell Research GMBH, Germany. Submitted by Hoechst A.G.
23. Beems RB and Til HP. Carcinogenicity study with Hoe 0-95 K in mice. Unpublished report of CIVO TNO (T10) No. R5058, 1976. Submitted by Hoechst A.G.
24. Sinkeldam EJ, van der Heiden CA and Koeter HBWM. Combined chronic toxicity and carcinogenicity study with Hoe 0-95 K in rats. Unpublished report of CIVO TNO (T4) No. R 5229, 1977. Submitted by Hoechst A.G.
25. Sinkeldam EJ, Kuper CF and Beems RB. Combined chronic toxicity and carcinogenicity study with Hoe 0-95 K in rats pretreated in utero. Unpublished report of CIVO TNO (T5) No. R6100, 1979. Submitted by Hoechst A.G.
26. Reuzel PGJ and van der Heijden CA. Long -term (two-year) oral toxicity study with Hoe 0-95K in Beagle dogs. Unpublished report of CIVO TNO, No. 5280, 1977. Submitted by Hoechst A.G.
27. Mukhopadhyay M, Mukherjee A and Chakrabarti J (2000). *In vivo* cytogenetic studies on blends of aspartame and acesulfame-K. Food and Chemical Toxicology 38:75-77.