ACESULFAME POTASSIUM

First draft prepared by Dr R. Walker,
Professor of Food Science, Department of Biochemistry, University of Surrey, England.

1. EXPLANATION

Acesulfame potassium (Acesulfame K) has previously been evaluated for an Acceptable Daily Intake by the Committee at the 25th and 27th meetings (Annex 1 references 56, 62). A toxicological monograph was prepared on the first occasion (Annex 1, 57) and a monograph addendum was prepared by the 27th Meeting (Annex 1, 63) when an ADI of 0-9 mg/kg b.w. was allocated based on a 2 year study in the dog in which the no-observed-effect-level (NOEL) of 900 mg/kg b.w. was the highest dose tested. In a two-year rat study, a higher NOEL of 1500 mg/kg b.w. was established.

Since the previous evaluation some new data have become available and are included in the following monograph. The previously published monograph and monograph addendum have been expanded and are included in their entirety. Further data on the potential breakdown products of Acesulfame K, acetoacetamide and acetoacetamide-N-sulfonic acid are also included.

In reviewing the new and previously published data as a whole, the Committee also considered whether the ADI might be increased and based on the second long-term rat study.

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

2.1.1.1 Rat

Single oral doses of 10 mg ^{14}C-Acesulfame K/kg b.w. given to rats and dogs were rapidly absorbed. Maximum blood levels reached were 0.75 ± 0.2 µg/ml in rats, 0.5 h after dosing, and 6.56 ± 2.08 µg/ml in dogs, 1-1.5 h after dosing. In rats, 82-100% of the dose, and in dogs, 85-100% of the dose was excreted in the urine; in both species, 97-100% of the total radioactivity was excreted in faeces, and total recovery approximated 100%.

Rats given 10 consecutive daily doses of 10 mg/kg orally did not show evidence of accumulation. Three days after dosing, the concentration in the organs and plasma was 0.4 nMol/g in liver, and less than 0.2 nMol/g in other tissues. Seven days after dosing, the concentration in dogs was less than 0.2 nMol/g in all tissues examined (Kellner & Christ, 1975a).

After intravenous administration of a single dose of 10 mg ^{14}C-Acesulfame K/kg b.w. to rats, the radioactivity was excreted quantitatively in urine and the plasma half-life was 0.23 h (Kellner & Christ, 1975a).

Single oral doses of approximately 15 mg ^{14}C-Acesulfame K/kg b.w. were administered to male and female rats which had been pretreated with unlabelled Acesulfame K at a level of 300 mg/kg diet for 60 days. Control animals without pretreatment were also similarly dosed with ^{14}C-Acesulfame K. In all animals 95.1-98.2% of the dose was recovered in urine and cage washings and 0.95-2.86% in faeces. Total recoveries were 96.3-99.2%. Excretion of radioactivity was rapid and displayed biphasic kinetics; 92.6-96.8% of the dose was excreted in 24 hours. The half-life of the rapid phase was 4-4.5 hours and of the slower phase (accounting for <0.5% of the dose) was 109-257 hours. No significant differences in route or rate of excretion were observed between sexes nor between controls and animals pretreated with Acesulfame K for 60 days (Volz & Eckert, 1981).

In a study of the pharmacokinetics after prior exposure to high doses, rats of both sexes were pretreated with 1% Acesulfame K in the diet for 7 days and then given a single oral dose of 500 mg ^{14}C-labelled compound; control groups were included without pretreatment. Urinary excretion was rapid, 89.7-93.8% of the dose was excreted in the first 24 h. The elimination was biphasic with estimated half-lives of 4 h for the rapid phase and 24-36 h for the slower phase. No significant differences were observed between the pretreated animals and controls. All the excreted radioactivity was in one peak which co-chromatographed with the parent compound and the experiment confirmed the lack of metabolism of Acesulfame K even after repeated administration of high doses (Volz *et al.,* 1983)

The absorption, distribution and excretion of ^{14}C-Acesulfame K was investigated by autoradiography in pregnant rats given a dose of 10 mg/kg b.w. on the 19th day of gestation. The kinetic behaviour in the dam was comparable with that in non-pregnant animals. The radioactivity in the fetuses at 0.5 h and 2 h after administration, when maternal blood levels were at their highest, was low; the ratio of activity in fetus and maternal blood was 1:14 and 1:3 at these two time points. The placentae contained higher concentrations than the fetus and the radioactivity in the amniotic fluid did not differ from background. The distribution in the fetus was uniform apart from somewhat higher activity in the glandular stomach (Kellner & Eckert 1983a).

In lactating rats given a single oral dose of ^{14}C-Acesulfame K of about 10.6 mg/kg b.w., activity was detected in the milk with a peak concentration occurring about 5 h after administration. The mean milk concentration over a 48 h period was about 6.3 times that in maternal blood. The biological half-lives were similar in milk (5.6 h) and blood (4 h). It was estimated that about 1.6% of the dose was eliminated in milk in the first 24 h and one-tenth of this on the second day (Kellner & Eckert, 1983b).

Following oral administration of a single dose of 3.6-4.5 mg ^{14}C-Acesulfame K/kg b.w. to pigs, maximum blood levels ranged between 0.35-0.72 µg/ml between 1-2 h after dosing and fell to undetectable levels within 48 h. Excretion occurred mainly in the urine (Kellner & Christ, 1975b).

2.1.2 Biotransformation

The metabolism of Acesulfame K was investigated in the urine and faeces of rats and dogs which had received single oral doses of 10 mg/kg b.w., and in the urine and bile of pigs dosed orally with 5 mg/kg b.w. The analytical methods used (thin-layer chromatography, mass spectrometry and isotope dilution) detected only the original substance in these samples (Volz, 1975).

Separation by TLC of urinary extracts from rats used in the above study revealed only one peak which was identical with Acesulfame K. No metabolites were detected in control or Acesulfame K-pretreated animals (Volz & Eckert, 1981). Similarly, no metabolites were detected in animals which had been pretreated with 1% Acesulfame K for 7 days (Volz et al., 1983).

2.1.3 Effects on enzymes

In vitro studies on acetoacetamide, a possible minor breakdown product of Acesulfame K, showed that it did not function as a substrate for thiolase, ß-hydroxyacyl-CoA-dehydrogenase, or ß-hydroxy-butyrate-dehydrogenase indicating that *in vivo* formation of acetamide is not probable (Anon., 1980b).

Investigation of the carbonic anhydrase-inhibiting effect *in vitro* showed that Acesulfame K had virtually no effect, concentrations of 180 mg/ml being required for 50% inhibition (Vogel & Alpermann, 1974).

2.2. Toxicological studies

2.2.1 Acute toxicity

Species	Route	LC_{50} (mg/l)	Reference
Zebra Fish	Water	>1000	Markert & Weigand, 1979a
Zebra Fish	Water	ca 2500[1] 1800-2500[2]	Markert & Jung, 1988
Golden Orfe	Water	>1000	Markert & Weigand, 1979b
Rat	p.o. i.p.	7430 2240	Anon. 1973 Mayer & Weigand, 1977

[1] after 48 hours
[2] after 96 hours

2.2.2 Short-term studies

2.2.2.1 Rat

Four groups of 10 male and 10 female weanling Wistar-derived rats were given diets containing 0, 1.0, 3.0, or 10% Acesulfame K for 90 days. Body weights were recorded weekly, food intake was determined during the first four weeks and in weeks 11 and 12. In week 13, the animals were bled from the tip of the tail and blood samples were examined from haemoglobin content, haematocrit, RBC and total differential white cell counts. Pooled urine samples from each group were collected in week 13 and examined for appearance, pH, glucose, protein, occult blood, ketones and microscopy of the sediment. At autopsy, blood samples were examined for SGPT, SGOT, alkaline phosphatase, total serum protein and serum albumin. Organ weights were recorded for heart, kidneys, liver, spleen, brain, testes/ovaries, thymus, thyroid, adrenals and caecum (filled and empty). Histological examination was carried out on haematoxylin/eosin sections of the weighed organs and on lung, salivary glands, trachea, aorta, skeletal muscle, axillary and mesenteric lymph nodes, pancreas, bladder, prostate, epididymis, uterus, mammary gland, oesophagus, stomach, duodenum, ileum and colon.

Food consumption of rats fed Acesulfame K at the 10% level was depressed during the first two to three weeks and body weight gain was markedly lowered during the first four weeks; slight diarrhoea and increased faecal water content occurred at this dose level. A slight increase in haemoglobin concentration was observed in males of the top dose group only, and total serum protein was slightly decreased in females only. Caecal enlargement was observed in both sexes receiving 10% Acesulfame K and in females receiving 3%. The relative weights of the liver and kidneys were slightly elevated in females of the 10% group and relative spleen weights were slightly depressed in all dose groups. Urinalysis, serum enzyme levels and serum albumin were not affected by the treatment, no gross pathological changes were detected, and no dose-related abnormalities were observed histologically (Sinkeldam, *et al.,* 1974).

These workers considered that the caecal enlargement was a physiological response to the presence of osmotically-active material in the gut and that, since liver, kidney and spleen weights were within the normal range of the strain of rat used, and no histological changes occurred, the no toxic effect level is conservatively placed at 3% in the diet; this is equivalent to 1.5 g/kg/day in rats.

2.2.2.2 Dog

Four groups of four female and four male beagle dogs, initially 17-21 weeks old, were fed diets containing 0, 0.3, 1.0, or 3.0% Acesulfame K for two years. Body weight was recorded weekly for the first 12 weeks and at

four-weekly intervals thereafter. Urinalysis, haematological examination and clinical chemistry were performed after 12, 26, 52, 78 and 104 weeks. Urinalysis included specific gravity, pH, sugar, protein, occult blood, ketone and microscopic examination of sediment; haematology comprised sedimentation rate, clotting time, haemoglobin, PCV, RBC count, WBC count and differential leucocyte count; clinical chemical investigations included blood sugar, urea, SGOT, SGPT, serum alkaline phosphatase, total serum protein and serum albumin. Liver function tests (bromosulfophthalein clearance) and kidney function tests (phenol red excretion) were performed on control and top dose group animals after 26, 52 and 104 weeks.

At termination, gross pathological examinations were performed and the following organs weighed: heart, kidneys, spleen, liver, lungs, testes/ovaries, thyroids, adrenals and brain. Histological examinations were performed on the weighed organs and also on the following tissues: spinal cord, sciatic nerve, salivary glands, skeletal muscle, thoracic aorta, skin, tonsils, axillary, superficial, cervical and mesenteric lymph nodes, bladder, oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, pancreas, trachea, circumanal glands, eyes, epididymis, prostate, uterus, gall bladder, tongue and thymus. A marrow smear (rib bone) was also examined. General appearance, condition, behaviour and survival were not affected by the treatment. None of the examinations performed revealed adverse effects related to the feeding of Acesulfame K. The no-toxic effect level was found to be higher than 3% in the diet, corresponding to an intake of 900 mg/kg/day in dogs (Reuzel & van der Heijden, 1977).

2.2.3 Long-term/carcinogenicity studies

2.2.3.1 Mouse

Four groups of 100 male and 100 female Swiss mice were fed diets containing 0, 0.3, 1.0, or 3.0% Acesulfame K for 80 weeks.

All survivors were sacrificed and autopsied, and weights of livers and kidneys were recorded. All tumours and tissues showing gross lesions suspected of being tumours and the livers of all animals were examined microscopically (haematoxylin and eosin sections). The feeding of Acesulfame K did not cause adverse effects on general appearance, behaviour or survival at any of the dietary levels but body weights were slightly decreased at the 3% dose level in both sexes. The relative liver weight was decreased at all dose levels in males only but there was no evidence of a dose-related response. Deaths occurring during the course of the study were attributed to chronic nephropathy, severe liver degeneration, respiratory infections and lung tumours. Gross and microscopic examination revealed a variety of tumours in both test and control animals, but evaluation of the data on type of tumour, location and incidence did not indicate that the test compound was carcinogenic to mice at dietary levels up to 3% for 80 weeks (Beems & Til, 1976).

2.2.3.2 Rat

A combined chronic toxicity and carcinogenicity study was performed on Wistar rats (CIVO strain) which were obtained from the $F1_a$ generation in a multigeneration study (see Special studies on reproduction, above). Four groups of 60 male and 60 female weanling rats were given diets containing 0, 0.3, 1.0, or 3% Acesulfame K for two years. The rats were derived from parents which had been maintained on the same diet since weaning. Periodic observations were made of appearance, behaviour, growth, and food intake. Haematological examinations were carried out after 13, 26, 52, 78 and 104 weeks, clinical chemical tests were performed on blood samples after 26, 52, and 104 weeks and urinalysis was done after 26, 52, 78, and 102 weeks. At termination, survivors were autopsied and organ weights recorded for heart, kidneys, spleen, liver, brain, gonads, thyroid, adrenals, and caecum (filled and empty). Tissue samples from 20 male and 20 female rats of the control and top dose groups only were subjected to comprehensive histological examinations; histology on other animals was limited to liver, spleen, adrenals, thyroid, parathyroid, pituitary and ovaries, and to grossly visible lesions suspected of being tumours. Body weight gain was decreased in both sexes of the top dose group during the first 44 weeks of the study but not significantly thereafter.

Death-rates of males fed 1.0 or 3% Acesulfame K and of females fed 0.3% Acesulfame K were higher than controls but it was considered that there was no evidence of mortality being increased by treatment, and the mortality of control rats was low for the strain of rat used. Interim deaths were mainly due to chronic respiratory disease and lymphoreticular malignancies of pulmonary lymphoid tissue. The incidence of pulmonary lymphoreticular tumours was relatively high in both males and females of the top dose group but only achieved statistical significance in females; there was also some evidence that these tumours appeared rather earlier in males of the mid and top dose groups. The results of haematological, clinical, chemical and urinalysis investigations were essentially normal in all dose groups. The relative weights of liver, kidneys, caecum and adrenals were increased in both sexes of the high dose group but the differences only reached statistical significance in (in males) liver and empty caecal weight and (in females) kidneys and caecal weight. Gross and histopathology did not reveal any treatment-related effects.

In commenting on these results, the authors pointed out the problems of inter-group comparisons in multigeneration studies where the animals in the different dose groups are not randomised. They stated that the increased death rate in test animals was still within the normal range for the strain of rats used and that the mortality in controls was lower than usual.

Pulmonary lymphoreticular tumours are a common cause of death in the strain of rats used, with very variable incidence, and the frequency in the test groups was within the normal range. This study concluded that their "higher" incidences and earlier appearance were fortuitous findings and did not suggest that Acesulfame K possessed carcinogenic properties (Sinkeldam *et al.,* 1977).

A second combined chronic toxicity and carcinogenicity study was carried out on a different rat strain with a lower incidence of pulmonary tumours in untreated animals. Four groups of 60 male and 60 female SPF-Wistar

rats received diets containing 0, 0.3, 1.0 or 3.0% Acesulfame K for 120-123 weeks. The rats used were progeny from parents which had been maintained on the same test diets since weaning. No adverse effects, other than decreased body weight in the top dose group, were observed in this study. In particular, there was no increased mortality or tumour incidence in the treatment groups. It was concluded that Acesulfame K failed to show carcinogenic or other effects of toxicological significance when fed to rats at levels of up to 3.0% for 120 weeks. (Sinkeldam *et al.,* 1979).

Following reservations about the extent of histological examination which had been carried out in this study, a detailed histopathological examination was performed on all animals in the control and top-dose groups, and in the lower (0.3% and 1%) dose groups. It was concluded that there were no treatment-related histopathological changes and, in particular, no evidence of an increase in the incidence or alteration of the biological type of the neoplasms diagnosed (Newman, 1982).

2.2.4 Reproduction studies

2.2.4.1 Rat

A multigeneration study in rats was carried out, in which males and females received Acesulfame K at dietary levels of 0, 0.3, 1.0 and 3.0% for three successive generations, each comprising two consecutive litters. A teratogenicity study was conducted with 15 females per group of the F_{2b} and F_{3a} generations. Rats from the F_{3b} generation were submitted to clinical and pathological examination. Pups from the F_{1a} litters were used for a chronic toxicity/carcinogenicity study at the same dietary levels of Acesulfame K as the parents (see Long-term/carcinogenicity studies). Fertility, number of young per litter, birth weight, growth rate and mortality during the lactation period were not adversely affected and there were no indications of increased mortality in utero. Growth rate was slightly decreased in the top dose group of the F_0 and F_1 generations, and the mid-dose group of the F_0 generation. In the teratogenicity studies, no adverse effects were seen in appearance, food consumption, autopsy of the dams, organ weights, or litter data; no visceral or skeletal abnormalities attributable to the treatment were observed.

In a four-week feeding study on rats of the F_{3b} generation, body weights and food efficiency were slightly decreased in males at the highest dose level. The relative weights of the caecum were slightly increased in both sexes of the high-dose group and in males of the mid-dose group. Gross and microscopic examination did not reveal any treatment-related pathological changes (Sinkeldam *et al.,* 1976).

In a separate study, Acesulfame K was fed to pregnant rats at dietary levels of 0, 0.3, 1.0, or 3.0% from day 6 up to and including day 15 of pregnancy; a positive control group received 75 000 i.u. vitamin A/rat/day during the same period. An increase in food consumption was observed at all three dose levels of Acesulfame K, most pronounced in the 0.3% group. Mean fetal weight showed a slight, dose-related increase in the test groups but skeletal and visceral examination of the fetuses revealed no teratogenic effects attributable to the feeding of Acesulfame K. A wide range of abnormalities was induced by teratogenic doses of vitamin A in positive controls (Koeter, 1975).

A reproduction study was carried out in which male and female rats were fed diets containing, 0, 0.3, 1.0, or 3.0% Acesulfame K for 12 weeks prior to mating; the dams received the same diet throughout pregnancy and lactation. Observations were made on the fertility of the females, number of young per litter, sex rates, gross abnormalities, mortality, body weight, and resorption quotient. Growth rate was slightly decreased in parent rats of the top dose group and in the mid-dose group females. No dose-related effects were seen in any of the observations made on the offspring, and there were no indications of increased mortality in utero. At weaning, 60 animals of each sex were selected from the litters for a two-year feeding study (see Long-term/carcinogenicity studies) (Sinkeldam, 1976).

2.2.4.2 Rabbit

An embryotoxicity study with Acesulfame K was carried out in which female rabbits received doses of 0, 100, 300 or 900 mg/kg bw by gastric intubation from the seventh to the nineteenth day after mating. On the twenty-ninth day of pregnancy, fetuses were delivered by Caesarean section; live and dead fetuses, resorptions and placentas were counted, weighed and examined macroscopically. The 24-hour survival was determined by incubation and half of the fetuses examined for skeletal abnormalities and the remaining half for visceral changes. One dam from the 300 mg/kg group had a premature birth. All other observations were within the range of control values and there was no evidence of compound-related malformations (Baeder & Horstmann, 1977).

2.2.5 Special studies on antigenicity

Acesulfame K was examined for potential antigenicity in an active systemic anaphylaxis test in guinea pigs. Groups of 10 male guinea pigs were challenged with Acesulfame K after sensitization with Acesulfame K plus Freund's adjuvant. Control groups were challenged with Acesulfame K after no prior treatment or after treatment with adjuvant alone. Positive control groups were challenged with bovine serum albumin. Acesulfame K showed no antigenic effect and only the animals sensitized with BSA showed anaphylactic reactions (Donaubauer *et al.,* 1987).

2.2.6 Special studies on caecal enlargement

These studies were performed to investigate the possible reversibility of caecal enlargement observed in short-term and long-term studies. Groups of 10 juvenile female Wistar rats, body weight approximately 115g, received Acesulfame K at dietary concentrations of 0, 3.0 or 10.0% for treatment period of 45, 49, and 90 days. One group from each dose level was sacrificed at the end of the treatment periods of 45 and 90 days; in

addition one group from each dose level was sacrificed after recovery periods of 41 days' treatment, and 14, 56 and 127 days following 90 days treatment. Food and water intake, and body weight were measured weekly.

At termination, the weights of caeca with and without contents, as well as moisture content of the contents, were determined. At the 10% feeding level, there was an increase in food and water intake and a reduction in body weight gain which was reversible during the post-treatment period. At this treatment level, filled caecal weight relative to body weight was approximately doubled after 45 and 90 days while at the 3% dietary level, a significant increase of about 30% was observed after 90 days only. A significant increase in the water content of caecal contents was observed only in the highest dose group. The changes in the caecal weights after 90 days exposure to 3% Acesulfame K in the diet were reversible within 14 days. After 90 days treatment at the 10% dietary level, the water content in the caecum returned to normal within 14 days but the filled caecal weight remained significantly increased (by approximately 30%) even after a recovery period of 127 days (Mayer *et al.,* 1978b).

A similar study was performed with adult female Wistar rats, body weight approximately 220 g. Initially, animals receiving 10% Acesulfame K in the diet experienced anorexia followed by an increased food consumption after two weeks. Water consumption was increased during the treatment period in both dose groups. Filled and empty caecal weights were increased by 80% and 33% respectively in animals receiving 10% Acesulfame K for 45 days. Increased caecal weights were observed in both dose groups after 91 days treatment. Caecal water content was significantly increased in both dose groups after 45 days but not after 91 days treatment. All the changes were reversible in animals of both dose groups treated for 40 days followed by a 42 day recovery period. After 91 days' treatment at the 3% dietary level, all changes were reversible within 14 days but filled caecal weights were still significantly increased (by about 30%) after a recovery period of 127 days (Mayer et al., 1978c).

These experiments did not reveal any significant differences between juvenile and adult animals with regard to induction or reversibility of caecal enlargement. Complete reversibility was demonstrated at the 3% dietary level, but not at 10% in the diet. The authors note that the coincidence of increased water intake with increased caecal water content and the reversibility of both of these parameters after withdrawal of Acesulfame K probably indicates that the changes are of osmotic origin.

2.2.7 Special studies on dermal irritation

Acesulfame K was non-irritant in a primary dermal irritation test in the rabbit (Kreilung & Jung 1988a).

2.2.8 Special studies in diabetic rats

Wistar rats of both sexes were rendered diabetic by a single i.v. dose of streptozotocin (60 mg/kg) 12 days prior to the commencement of a short-term study. Groups of 20 male and 20 female rats were selected from the diabetic animals, only those with blood glucose levels greater than 300 mg% being used, and received Acesulfame K in the diet at levels of 0 (control), 0.3, 1.0 and 3.0% for 28 days. General condition, food and water intake and body weight gain were recorded and blood glucose levels were determined at weekly intervals; urinalysis was performed weekly.

At termination, haematological and clinical biochemical analyses were carried out, organ weights determined and gross and histological examinations were performed. No differences in general condition, behaviour and food consumption were noted between diabetic controls and treated animals; water consumption was higher in groups fed Acesulfame K, possibly as a result of the osmotic effect of the high dietary load. All other observations, including histology, did not reveal any differences due to treatment with Acesulfame K (Mayer & Kramer, 1980).

2.2.9 Special studies on DNA binding

After pretreatment for seven days with a diet containing 3% Acesulfame K, male rats were given a dose of 250 mg Acesulfame K containing ^{14}C-Acesulfame K (9.6 x 108 dpm) by oral gavage. After eight hours the animals were killed and liver and spleen excised; DNA and chromatin protein was isolated from these organs. No radioactivity could be detected on any DNA sample. A low level of activity (8-11 dpm/mg protein) was associated with chromatin protein and this was claimed to be due to non-covalent interactions of unchanged Acesulfame K (Sagelsdorff *et al.,* 1981).

2.2.10 Special studies on genotoxicity

Table 1

Test System	Test object	Concentration	Result	Reference
Mouse micronucleus assay	Male and female mice	2x450-4500 mg/kg b.w	Negative	Baeder & Horstmann, 1977
Dominant lethal assay	Male rat	1-3% diet	Negative	Willems, 1974
Chromosome aberration test	Chinese hamster bone marrow	450-4500 mg/kg b.w.	Negative	Mayer et al., 1978a
In vitro mammalian gene mutation (8-	Chinese hamster V79 cells	10-10000 µg/ml	Negative	Marquardt, 1978

azaguanine resistance)				
Cell transformation	M2 mouse fibroblast cells	10-10000 µg/ml	Negative[1]	Marquardt, 1978
Unscheduled DNA synthesis	Primary rat hepatocytes	25-10000 µg/ml	Negative[1]	Myhr & Brusick, 1982
Ames test[2]	S. typhimurium TA98, TA100, TA15325, TA1537	0-100 mg/plate	Negative	Gericke, 1977
Ames test[2]	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	4-5000 µg/plate	Negative	Jung & Hollander, 1986
	E. Coli WP2uvrA	4-5000 µg/plate	Negative	
Ames test[2]	S. typhimurium TA98, TA100 TA1535, TA1537	0-100 mg/plate	Negative	Rohrborn, 1976

[1] Cytotoxicity at 5000-10 000 µg/ml
[2] In the presence or absence of rat liver S9 fraction

2.2.11 Special studies on primary eye irritation

Acesulfame K was examined for primary irritation in the eye of New Zealand white rabbits. From 1 to 72 hours after application the treated eyes of all animals showed injection of conjunctival vessels to a diffuse beefy red colour and there was swelling of lids. The conjunctivae and nictating membrane were blanched. At the 1 and 24 hour examinations, the irises were reddened and there was colourless discharge. From 48 h to 7 days some haemorrhage of the conjunctiva and nictating membranes occurred but all signs of irritancy had reversed by day 14 (Kreilung & Jung, 1988b).

2.2.12 Special studies of the effects on bacteria

Acesulfame K was without antibacterial activity against 12 bacterial strains *in vitro,* and did not show antibacterial activity in experimental septicaemia in the mouse with *Streptococcus pyogenes* A77 or *Salmonella typhimurium.* Long term culture (30 daily passages) of *Staphylococcus aureus* and *E. coli* with a range of concentrations of Acesulfame K did not affect growth characteristics nor sensitivity towards antibiotics, ampicillin, cephalothin, tetracycline or gentiamycin (Schrinner & Limbert, 1977).

2.2.13 Special studies on nitrosation of Acesulfame K

In vitro studies were performed to investigate whether Acesulfame K could form N-nitroso derivatives. Nitrosation was carried out using N_2O_3 in glacial acetic acid, or excess $NaNO_2$, at pH 3 and pH 1. An N-nitroso compound was detected in low yield with N_2O_3 or with nitrite at pH 1 but not at pH 3. The yield at pH 1 was estimated to be 0.4×10^{-3}% (Eisenbrand, 1979). Acesulfame K was incubated at 37°C with excess $NaNO_2$ (276 mg/10 ml) at pH 3 and pH 1 for one hour and four hours. The maximum yield of N-nitroso derivatives was 1.4×10^{-3}% after four hours at pH 1. This was considered to represent a negligible hazard *in vivo* (Eisenbrand, 1982).

2.2.14 Special studies on pharmacological aspects

2.2.14.1 In vitro

No functional changes were detected after application of 50 mg Acesulfame K to isolated guinea-pig heart using the Langendorff techniques; the compound did not show antiarrhythmic activity in isolated, perfused guinea-pig heart with aconitine and digitoxin-induced fibrillation. In the isolated ileum of guinea-pig, Acesulfame K at a concentration of 10 mg/ml had no neurotropic or spasmolytic effect on smooth muscle. No functional changes were detected after application of 50 mg Acesulfame K to isolated guinea-pig heart using the Langendorff techniques; the compound did not show antiarrhythmic activity in isolated, perfused guinea-pig heart with aconitine and digitoxin-induced fibrillation.

In the isolated ileum of guinea-pig, Acesulfame K at a concentration of 10 mg/ml had no neurotropic or spasmolytic effect on smooth muscle. Addition of Acesulfame K to dog plasma *in vitro* was without effect on thrombin time, thromboplastin time or recalcification time (Vogel & Alpermann, 1974).

2.2.14.2 Mouse

Dosages of Acesulfame K of 400 mg/kg i.p., 500 mg/kg orally, or 320 mg/kg subcutaneously, did not depress motor activity of mice excited by Pervitin. The hexobarbital sleeping time in mice was not changed by pretreatment with Acesulfame K at doses of 500 mg/kg *per os* or 160 mg/kg subcutaneously. Metrazol-induced convulsions in mice were not influenced by Acesulfame K at doses of 500 mg/kg *per os,* 300 mg/kg i.p., or 320 mg/kg subcutaneously; anti-convulsant activity can thus be excluded. Administration of Acesulfame

K (200 mg/kg i.p.) to mice was without effect on tetrabenazine-induced ptosis and catalepsy, thus the compound is without anti-depressant properties. Acesulfame K (200 mg/kg i.p., 160 mg/kg s.c., or 500 mg/kg *per os*) was without effect on compulsive gnawing behaviour induced by the combined application of apomorphine and imipramine, therefore antidepressant and anticholinergic effects are unlikely.

At doses of 320 mg/kg s.c. or 500 mg/kg orally, Acesulfame K had no analgesic effect on mice (Vogel & Alpermann, 1974).

2.2.14.3 Rat

Predosing of rats with Acesulfame K (500 mg/kg orally or 160 mg/kg s.c.) was without anti-inflammatory effect on Aerosil-induced paw oedema, and similar doses had no antipyretic effect in rats with yeast-induced fever. Eight daily doses of Acesulfame K (0-100 mg/kg) *per os* had no effect on serum cholesterol, total glycerol, free glycerol or glucose levels; relative liver weights were unchanged. In acute tests, Acesulfame K had no effect on blood sugar levels in rats given 100 mg/kg orally, guinea-pigs given a similar dose i.p., or rabbits receiving 100 or 500 mg/kg orally or 50 mg/kg i.v. Acesulfame K had no diuretic effect in rats and dogs at oral dose levels of 50 mg/kg and 20 mg/kg respectively (Vogel & Alpermann, 1974).

2.2.14.4 Guinea pig

Acesulfame K given intravenously to guine-pigs at a dose level of 24 mg/kg reduced digoxin toxicity. This effect was due to the potassium content and not to antiarrhythmic activity of the compound. Intravenous administration of 1-5 mg Acesulfame K/kg to anaesthetised guinea-pigs one minute before treatment with histamine was without effect on the bronchial musculature (Vogel & Alpermann, 1974).

2.2.14.5 Dog

Cardiovascular experiments in anaesthetized dogs showed that intravenous administration of Acesulfame K was without effect up to a dose of 6 mg/kg; doses of 12 and 24 mg/kg caused a decrease in contractility of the heart with a transient reduction of blood pressure and peripheral blood flow. The changes were compensated for in 3-5 minutes. Intraduodenal administration of Acesulfame K to anaesthetized dogs at dose levels of 0-1000 mg/kg induced a slight reduction of blood pressure at 500 mg/kg and this was accompanied by a reduction of cardiac contractility of about 20% at 1 g/kg. The effect was reversed in 50-80 minutes and other cardiovascular parameters were unchanged. In the conscious dog, after a five day treatment with Acesulfame K at 100 mg/kg *per os* daily, no change in blood pressure or cardiac activity could be detected. Acesulfame K at doses of 0-24 mg/kg i.v. had no antiarrhythmic effect on anaesthetised dogs poisoned with K-strophanthin. Daily oral administration of Acesulfame K (1 g/kg) to dogs for 14 days was without effect on thromboplastin time, thrombin time, recalcification time and thromboelastography of plasma samples (Vogel & Alpermann, 1974).

2.2.15 Special studies on the possible reactions of Acesulfame K with food constituents.

Acesulfame K (1% aqueous solutions) was heated at 100 °C with the model food constituents, ethanol, sorbitol, glycine, alanine, glutamic acid, phenyl alanine or n-butylamine, in acetate buffer at pH 5. Analysis by HPLC and UV-spectrometry failed to detect any decomposition or interaction products of Acesulfame K in any of the model systems (Clauss, 1981).

2.2.16 Special studies on thermal degradation products of Acesulfame K - inhalation studies.

Two groups of 5 male and 5 female rats were exposed to the products of pyrolysis of Acesulfame K at 250 °C in an inhalation chamber. Some disturbances in respiration (irregular/intermittent breathing) and nasal discharge were noted during exposure but all animals survived for an observation period of 14 days. No abnormal signs were seen at autopsy at the end of the experiment (Hollander & Weigand, 1986).

2.2.17 Special studies on acetoacetamide.

Acetacetamide may be formed to a very small extent during long-term incubation of Acesulfame K in fluids of low pH. A number of studies have been conducted on this substance.

2.2.17.1 Absorption, metabolism, distribution and excretion.

The pharmacokinetics of acetoacetamide were studied in rats after a single oral or i.v. dose of ^{14}C-acetoacetamide, or after 10 daily oral doses of 1 mg/kg b.w. The compound was rapidly absorbed, maximal blood levels being achieved 0.5-1 h after oral administration. Subsequent excretion was biphasic with half-lives of 2.7 ± 0.3 h and 99 ± 28 h; similar excretion kinetics were observed after i.v. dosing. After repeated dosing, some cumulation of radioactivity in the blood was observed. Within 7 days after administration of a single dose, rats (both sexes) excreted 90-97% (oral dose) or 93-98% (i.v. dose) of the radioactivity in urine and faeces, urine alone accounting for more than 90% in both cases. About 9.3% of orally administered radioactivity was in expired air. On repeated oral dosing, excretion of activity was uniform in urine and faeces and amounted to 90% after 24 h following the 10th day of administration, of which about 85% was in urine (Eckert & Kellner, 1979).

Three human volunteers were given oral doses of 50 mg doubly isotopically labelled acetoacetamide. Absorption was rapid, peak blood levels being achieved between 15 min and 3 h after ingestion. Elimination was rapid, $t_{1/2}$ 9.7 ± 0.22 h, more than 95% being excreted in urine and less than 1% in faeces (Eckert *et al.,* 1980).

The metabolism of acetoacetamide was studied after single and repeated oral administration of different doses to hamster, rat, rabbit, dog and man. Rapid urinary excretion occurred in all species and TLC analysis of urine revealed the presence of 5 peaks. Apart from the original compound, the following metabolites were identified: ß-hydroxybutyramide, erythro- and threo-2,3-dihydroxybutyramide. In addition, with some variation with species, the presence of oxamic acid, malic acid amide and a conjugate of 2,3-dihydroxybutyramide were tentatively identified and, at least in rats, some of the dose was respired as CO_2. The rabbit was most similar to humans in the metabolism of acetoacetamide (Volz & Fehlhaber, 1981).

2.2.17.2 Acute toxicity of acetoacetamide

The oral LD_{50} of acetoacetamide in female rats was greater than 15 g/kg b.w. (Anon., 1977). No gross adverse effects other than a slight diarrhoea were seen in one male and one female beagle dog after administration of 5 g/kg b.w.

2.2.17.3 Short-term studies on acetoacetamide

2.2.17.3.1 Rat

In a preliminary range-finding study, groups of 5 male and 5 female Wistar rats were given acetoacetamide in the diet at concentrations of 0 or 5% for 29 days. There was an impairment of body weight gain in the treated group from day 8 and at termination the deficit of weight relative to controls was 31% and 26% in treated males and females respectively. Haematological examinations revealed an increased leucocyte count in treated males and a significant drop in RBC and haemoglobin levels in both sexes. Urinalysis and clinical chemistry were essentially normal except for a reduction in serum alkaline phosphatase. Some significant changes were observed in relative organ weights but in view of the large difference in body weight, the relevance of these is unclear. At autopsy, the livers of most treated animals appeared abnormal and some animals had enlarged thyroids. Histological examination showed marked hepatic centrilobular fatty degeneration. The dose tested was in excess of the no observable effect level (Mayer *et al.,* 1978e).

Acetoacetamide was administered to groups of 15 male and 15 female rats for 90 days in the diet at concentrations of 0, 400, 2000, 10 000 and 50 000 mg/kg. Body weight gains and feed consumption were recorded throughout and haematological examinations and urinalysis were carried out at termination; clinico-chemical analyses were performed in the middle of the study and at termination.

At autopsy, major organs were weighed and histological examinations were conducted. Behaviour and condition were unaffected by treatment but there was a reduction in weight gain of both sexes at the highest dose level; at the 10 000 mg/kg level there was a transient reduction in weight gain in males only. Erythrocyte counts and haemoglobin were depressed in both sexes at the top dose level; the RBC was also decreased in males of the 10 000 mg/kg dose group. Clinico-chemical parameters and urinalysis results were generally unremarkable except for a transient decrease in serum alkaline phosphatase in males of the highest dose group. Histology revealed fatty degeneration in livers at the top dose level and thyroid changes *(Stroma parenchymatosa)* at the 10 000 and 50 000 mg/kg levels. The no observed adverse effect level was found to be 2000 mg/kg in rat diet (Mayer *et al.,* 1979).

2.2.17.3.2 Rabbit

Four groups of 6 male and 6 female Albino Himalayan rabbits were given acetoacetamide in drinking water for 90 days at concentrations of 0, 1200, 6000 or 30 000 mg/l. Behaviour and general condition of rabbits in the low and middle dose groups remained normal throughout but animals in the top dose group showed apathy and body weight gain was impaired, especially in males. In all groups food and drinking water consumption were not affected and haematological and urinalysis parameters remained normal. Clinical chemical analysis was similarly unremarkable except for a significant decrease in serum alkaline phosphatase in animals of both sexes, an increase in BUN in females and of uric acid in males of the highest dose group only.

At autopsy, organ weights were normal except for increased thyroid and decreased testis weights confined to the highest dose group. Histological examination revealed marked thyroid changes, interpreted by the authors as activation, in rabbits of the highest dose group and male rabbits in this group showed disorders of spermatogenesis. No such effects were seen in the low and middle dose groups and the no observed effect level was established at 6000 mg/l in drinking water, corresponding to a mean daily dose of about 500 mg/kg b.w. (Hollander *et al.,* 1981).

2.2.17.3.3 Dog

Four groups of two male and two female English beagle dogs were given acetoacetamide at doses of 0, 100, 500 or 2500 mg/kg b.w. by gastric intubation on 14 consecutive days. Haemoglobin, haematocrit and RBC counts were lowered at the highest dose level in both sexes and there was an increase in reticulocytes and Heinz bodies. Clinical-chemical examinations showed a clear increase in total bilirubin and creatinine in the top dose group and glucose levels were slightly lowered relative to controls. There was an increase in alkaline phosphatase in both sexes at the 100 mg/kg dose. Histological examination showed that there were thyroid changes at all dose levels and, at the highest level, there was increased iron accumulation in hepatic Kupffer cells. It was concluded that the no observed effect level for acetoacetamide in the dog is less than 100 mg/kg b.w. (Mayer *et al.,* 1980).

2.2.17.4 Genotoxicity of Acetamide

Table 2

Test system	Test object	Concentration	Result	Reference
Ames test[1]	S. typhimurium TA98, TA100 TA1535, TA1537	0-100 mg/plate	Negative	Gericke, 1977
In vitro mammalian gene mutation (8-azaguanine resistance)	Chinese hamster V79 cells	10-10000 µg/plate	Negative	Marquardt, 1978
In vitro mammalian gene mutation (8 – thioguanine resistance)	Chinese hamster V79 cells	100-1011 µg/ml	Negative	Müller, 1989a
In vitro chromosome aberration test[1]	Chinese hamster V79 cells	101-1011 µg/ml	Negative	Müller, 1989b
Cell transformation	M2 mouse fibroblast cells	10-10000 µg/ml	Negative	Marquardt, 1978
Unscheduled DNA synthesis	Human cell line A549	1-1000 µg/ml	Negative	Müller, 1989c

[1] In the presence or absence of rat liver S9 fraction.

2.2.17.5 Pharmacological studies

2.2.17.5.1 Mouse

Doses up to 250 mg/kg b.w. did not induce behavioural changes in mice. Pretreatment with acetoacetamide did not affect hexobarbital sleeping time and was without effect on tetrabenazine-induced ptosis in mice. Oral or sub-cutaneous acetoacetamide had no inhibitory effect on pentylenetetrazole-induced extensor convulsions in mice; clonic convulsions and survival time were unaffected. Oral or s.c. acetoacetamide at doses up to 250 mg/kg did not protect against electric-shock-induced extensor convulsions and had no analgesic effect in the tail-flick test in mice (Alpermann & Scholtholt, 1979).

2.2.17.5.2 Rat

Acetoacetamide had no diuretic effect in the Lipschitz test in water-loaded rats and did not have a saluretic effect in oral doses between 10 and 250 mg/kg b.w. *per os* or s.c. Acetoacetamide had no effect on blood glucose in glucose loaded rats. At doses up to 250 mg/kg b.w. orally or s.c. acetoacetamide had no anti-inflammatory activity (carrageenan-induced paw oedema test) and had no antipyretic effect in rats. Acetoacetamide did not exhibit alpha-adrenolytic activity in the isolated vas deferens of the rat (Alpermann & Scholtholt, 1979).

2.2.17.5.3 Guinea pig

Acetoacetamide (10 mg/kg b.w. i.v.) did not antagonize histamine-induced bronchoconstriction in guinea pigs. *In vitro,* acetoacetamide did not affect contractile force or heart rate in isolated guinea pig atrium and, at a concentration of 10^{-5} g/ml had no effect on contractions in isolated guinea pig ileum induced by barium chloride, carbachol or histamine. Acetoacetamide similarly was without effect on carbachol-induced contractions of isolated guinea pig trachea. (Alpermann & Scholtholt, 1979).

2.2.17.5.4 Dog

Intravenous doses of 10 or 25 mg/kg, or an intraduodenal dose of 250 mg/kg b.w., did not induce acute changes in cardiovascular parameters in the dog (Alpermann & Scholtholt, 1979).

2.2.18 Special studies on ß-hydroxybutyramide - mutagenicity.

ß-Hydroxybutyramide, a metabolite of acetamide in some mammalian species, was non-mutagenic in the Ames test against four strains of *Salmonella typhimurium,* with and without metabolic activation (Engelbart, 1979).

2.2.19 Special studies on acetoacetamide-N-sulfonic acid.

Acetoacetamide-N-sulfonic acid is formed to a small extent when Acesulfame K is incubated at low pH and is thus a potential minor contaminant. A number of studies have been carried out on this compound.

2.2.19.1 Absorption, distribution, metabolism and excretion.

The kinetics and metabolism of acetoacetamide-N-sulfonate, sodium salt, were studied in male rats after single oral or i.v. doses of 10 mg/kg b.w. Maximum blood levels occurred two hours after oral dosing (1.33 µg equiv./ml) and subsequent elimination was biphasic with half-lives of approx. 1 and 5 hours. After i.v. dosing elimination was rapid and predominantly renal (69.6 ± 9.2%) whereas after oral administration, faecal excretion predominated (55.2 ± 6.9%). After 7 days less than 0.06% of the radioactivity remained in the organism. Distribution studies showed higher levels in kidneys, urinary bladder and smooth muscle than in blood; after 24 hours concentrations in the tissues were generally below 0.1 µg equiv./g except in adrenals (0.13 µg/g), bladder (0.14 µg/g) and retro-peritoneal fat (0.15 µg/g). Incubation with faecal microflora *in vitro* resulted in complete decomposition of sodium acetoacetamide-N-sulfonate, two products being detected, one of

which was tentatively identified as ß-hydroxybutyramide-N-sulfonic acid. *In vivo,* besides the starting material, up to four metabolites were detected but not identified in the urine of male and female rats (Gross *et al.,* 1987).

2.2.19.2 Toxicological studies

2.2.19.2.1 Acute toxicity studies

Species	Sex	Route	LD_{50} (mg/kg b.w.)	Reference
Rat	M&F	oral	>5000	Rupprich & Weigand, (1984a)
Rat	M&F	i.v.	>3150	Rupprich & Weigand, (1984b)

2.2.19.2.2 Short-term studies

2.2.19.2.2.1 Rat

Acetoacetamide-N-sulfonic acid, sodium salt, was administered by gavage to Wistar rats at a dose of 1000 mg/kg b.w. daily, five days per week for 4 weeks. Behaviour, general health, food and water consumption and weight gain were unaffected. Haematological, clinical chemical and urinalysis examinations did not indicate any toxic changes. No adverse effects were seen at gross autopsy nor on histological examination. It was concluded that the no adverse effect level was over 1000 mg/kg b.w. (Hollander *et al.,* 1985).

Groups of 30 male and 30 female rats were given acetoacetamide-N-sulfonic acid in the diet at levels of 0, 8000, 20 000 or 50 000 mg/kg diet for three months. Ten animals of each sex/group were used in a recovery experiment. No changes due to treatment were observed in the 8000 mg/kg group during the in-life phase; the two higher dose groups showed slightly reduced body weight gain and slightly lower serum glucose which the authors attributed to limited carbohydrate availability due to a mild diarrhoea in these groups. The two highest dose groups showed slightly higher serum bilirubin and calcium values but these were only significant in males from the 20 000 mg/kg group. In addition, animals from the highest dose group displayed a mild anaemia. Urinalysis showed lower pH values and higher specific gravity for animals in the two highest dose groups and these displayed higher kidney weights, probably reflecting the increased renal excretion of the test compound. Animals in the two highest dose groups showed caecal enlargement, and histological examination showed a reversible hyperplasia of the rectal lymph follicles. In the absence of other histological signs and in the absence of anaemia, the authors concluded that the no adverse effect level was 20 000 mg/kg diet, corresponding to a mean daily dose of 1863 mg acetoacetamide-N- sulphonic acid/mg b.w. (Fuchs *et al.,* 1986).

2.2.19.2.2.2 Dog

Four groups of two male and two female beagle dogs were given acetoacetamide-N-sulphonic acid in the feed at concentrations of 0, 3750, 7500 or 15 000 mg/kg for two weeks. All animals survived and there were no clinical signs with the 3750 and 7500 mg/kg groups; administration of 15 000 mg/kg caused diarrhoea. No other reactions or changes attributable to treatment were detected on gross or histological examination; one female from the top dose group displayed a meningo-encephalitis which was not attributed to treatment (Brunk *et al.,* 1986).

2.2.19.2.2.3 Monkey

In a preliminary tolerance study, six Cynomolgus monkeys (3 males and 3 females) were given daily oral doses of 1000 mg/kg b.w. acetoacetamide-N-sulfonic acid for thirty days. Diarrhoea of very slight to moderate intensity was observed from day 6 but food consumption and body weight were not affected. The results of electrocardiographic and ophthalmoscopic examinations were normal and haematology, blood chemistry and urinalysis did not reveal any treatment related abnormality. Macroscopic and histological examinations at termination did not show any changes related to treatment. It was concluded that the dose of 1000 mg/kg b.w. did not elicit any systemic toxicity and, apart from the sporadic diarrhoea, the substance was well tolerated (Read *et al.,* 1989b).

Four groups of 4 male and 4 female cynomolgus monkeys were given acetoacetamide-N-sulfonic acid orally at dose levels of 0, 100, 315 and 1000 mg/kg b.w. daily for 13 weeks. Diarrhoea was observed episodically in one male from the 100 mg/kg group and chronically in 3 females and all males from the 315 mg/kg group and all animals in the top dose group. The onset of diarrhoea occurred 3-7 h after dosing. Body weight and food consumption were unaffected by treatment and ophthalmoscopic examination in the final week did not reveal any treatment-related abnormalities. Haematological, clinical chemical and urinary parameters were not affected by treatment and gross and histo-pathology were unremarkable. It was concluded that, apart from the digestive tract disturbances indicated by diarrhoea, acetoacetamide-N-sulfonic acid was well tolerated and no systemic toxicity was observed (Read *et al.,* 1989a).

2.2.19.2.3 Genotoxicity of acetoacetamide-N-sulfonic acid

Table 3

Test System	Test Object	Concentration	Result	Reference
Mouse micronucleus assay	Male and female mice	5000 mg/kg b.w.	Negative	Mayer & Weigand, 1985
Unscheduled DNA	Human cell line	1-1000 µg/ml	Negative	Müller, 1988a

synthesis[1]	A549			
In vitro mammalian gene mutation (6-thioguanine resistance)	Chinese hamster V79 cells	0-2000 µg/ml	Negative	Müller, 1988b
In vitro chromosome aberration test[1]	Chinese hamster V79 cells	0-2000 µg/ml	Negative	Müller, 1989d
Ames test[1]	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	4-5000 µg/plate	Negative	Müller, 1989c
	E.Coli WP2uvrA	4-5000 µg/plate	Negative	

[1] In the presence or absence of rat liver S9 fraction

2.2.19.2.4 Pharmacological aspects

2.2.19.2.4.1 In vitro

Acetoacetamide-N-sulfonic acid had no effect on the force or rate of contraction of the isolated guinea pig right atrium, and contractions initiated by carbachol, histamine or barium chloride in the isolated guinea pig ileum were unaffected at a concentration of 10^{-5} g/ml. This compound had no relaxant effect on isolated guinea pig trachea and was without alpha-adrenolytic effect on isolated vas deferens of the rat (Scholtholt & Alpermann 1984).

2.2.19.2.4.2 Mouse

Acetoacetamide-N-sulfonic acid at oral or s.c. doses up to 250 mg/kg b.w. did not cause behavioural changes in mice and pretreatment with this compound had no effect on hexobarbital sleeping time. Acetoacetamide-N-sulfonic acid had no effect on tetrabenazine-induced ptosis. It did not inhibit the extensor spasms induced by pentetrazole; clonic spasms and survival time were unaffected. Oral or s.c. doses of up to 250 mg/kg b.w. were without protective effect against extensor spasms initiated by electric shock and were without analgesic effect in the heat pain test (Scholtholt & Alpermann, 1984).

2.2.19.2.4.3 Rat

Acetoacetamide-N-sulfonic acid had no diuretic effect in the Lipschitz test in rats and oral or s.c. doses of 10 to 250 mg/kg b.w. had no saluretic effect. The compound was without effect on blood sugar in glucose tolerance tests. It had no anti-inflammatory effect in the carrageenan-induced rat-paw oedema test at doses up to 250 mg/kg b.w. *per os* or s.c. and showed no antipyretic effect (Scholtholt & Alpermann, 1984).

2.2.19.2.4.4 Guinea pig

Acetoacetamide-N-sulfonic acid at doses of 1-10 mg/kg b.w. i.v. had no antagonistic effect on histamine induced broncho-constriction in the guinea pig (Scholtholt & Alpermann, 1984).

2.2.19.2.4.5 Dog

No acute changes in cardiovascular performance parameters were observed in dogs given acetoacetamide-N-sulphonic acid at doses of 10 or 25 mg/kg b.w. i.v. or 250mg/kg intraduodenally (Scholtholt & Alpermann, 1984).

2.2.19.3 Human studies

Six healthy male volunteers received a dose of 50 mg/kg acetoacetamide-N-sulfonic acid. No treatment-related adverse reactions were reported and blood pressure and heart rate did not show any abnormal changes. Occasional, individual borderline changes in some haematological or clinical chemical parameters were not considered by the authors to be of clinical significance and most parameters were within normal ranges. It was concluded that this single dose was safe and well tolerated (Rosenkrantz *et al.,* 1988).

2.2.20 Special studies on the short-term toxicity of potassium chloride

Feeding studies in rats with potassium chloride were conducted to elucidate the possible involvement of potassium ion on Acesulfame K in changes observed in toxicological studies. Three groups of 20 male and 20 female weanling Wistar rats were fed for 90 days on diets containing 0, 12 000 or 37 000 ppm (0, 1.2 or 3.7%) KCl, equivalent to the potassium content of diets containing 0, 3, or 10% Acesulfame K. Regular measurements were made of body weight, food and water consumption, urinary volume and potassium content. After the animals had been sacrificed, the caeca with and without contents were weighed and the water contents of the caecal contents were determined. There was a dose-related increase in water consumption in both sexes through the study and, in the first few weeks, the food consumption of treated animals was slightly lower than that of controls. Body weight gains were depressed in males of both concentration groups throughout the 90 day period but in females statistically significant differences were obtained only up to the twenty-ninth day. Dose-related increases in urine volumes and urinary potassium were observed. Filled caecal weights were increased by about 10% in males and 20% in females of the top dose group, but these differences were not statistically significant; no differences were observed in empty caecal

weights, nor in water content of the caecal contents. It was concluded that the potassium content of Acesulfame K could be responsible for some adverse effects seen in toxicological studies; in particular depressed body weight gain (Mayer et al., 1978d).

2.3 Observations in man

Three human volunteers, body weight 70-80 kg, were given a single oral dose of 30 mg ^{14}C-Acesulfame K in peppermint tea. Absorption was rapid and virtually complete, maximum blood concentrations of 0.28 mg/ml occurring after 1 to 1-1/2 h. Elimination occurred rapidly with a plasma half-life of 2-1/2 h. Over 99% of the dose was excreted in urine and less than 1% in faeces; 98% of the activity was eliminated in the first 24 h. From the pharmocokinetic data it was calculated that repeated doses of 30 mg at 3 h intervals would increase the maximum serum levels 1.7-fold and at 2 h intervals maximum serum levels would increase 2.4-fold relative to a single dose (Christ & Rupp, 1976). The metabolism of Acesulfame K was studied in serum and urine from human volunteers following a single dose of 30 mg per individual. Only the original substance was detected in all samples (Volz, 1976).

3. COMMENTS

The Committee reviewed further data which confirmed the validity of the earlier long-term study in rats and the no-observed- effect level. A review of comparative pharmacokinetic data in rats and dogs showed that the blood levels of acesulfame potassium reached after similar doses were higher in dogs; there was no evidence to suggest that in relation to blood levels the dog was more sensitive to the effects of the substance than the rat.

Pharmacokinetic studies in humans showed that oral doses of acesulfame potassium were completely absorbed and rapidly excreted unchanged in the urine. The half-life in the plasma was 1.5 hours, which indicated that the period of exposure to the substance was brief and no accumulation occurred.

Since acesulfame potassium was not metabolized in any species tested, including humans, and further studies in rats in which repeated doses were given did not reveal any induction of metabolism or change in pharmacokinetic behaviour, the Committee concluded that the rat appeared to be an appropriate model for humans. Consequently, the Committee decided that, since the 2-year study in rats represented a greater proportion of the lifespan of the species than did the 2-year study in dogs and included exposure to the substance *in utero,* the ADI should be based on the no-observed- effect level in the rat, i.e., 1500 mg/kg of body weight per day. The Committee also noted new data which indicated that acesulfame potassium had no adverse effects in diabetic rats and was not allergenic in an active systemic anaphylaxis test in guinea pigs.

The Committee also reviewed extensive toxicological studies on the breakdown products, acetoacetamide and acetoacetamide- *N*- sulfonic acid, which indicated that these compounds have a low toxicity and are not mutagenic.

In view of these data and of available estimates of exposure to acesulfame potassium, the Committee concluded that acetoacetamide- *N*-sulfonic acid and acetoacetamide did not represent a health hazard under present or foreseeable conditions of use of acesulfame potassium.

4. EVALUATION

The previously established ADI was changed to 0-15 mg/kg b.w. based on the long-term study in the rat.

The toxicological evaluation was carried out based on the existing specifications which should be reviewed and revised in the near future taking into account information on current manufacturing purification procedures.

5. REFERENCES

ALPERMANN, H. & SCHOLTHOLT, J. (1979) Pharmacology of aceto-acetamide. Unpublished report dated 12th June, 1979. Submitted to WHO by Hoechst A.G.

ANON. (1973). 3,4-Dihydro-6-methyl-1,2,3-oxothiazine-4-one-2, 2-dioxide potassium. Determination of acute oral toxicity in female SPF-Wistar rat. Unpublished report. Submitted to WHO by Hoechst A.G.

ANON. (1977). Acute oral toxicity of acetamide in female SPF Wistar rats. Report No.427/77. Unpublished report. Submitted to WHO by Hoechst A.G.

ANON. (1980). Investigating the efficacy of acetoacetamide as a substrate of ß-hydroxyacyl-CoA-dehydrogenase and ß-hydroxy-butyrate-dehydrogenase. Unpublished report. Submitted to WHO by Hoechst A.G.

BAEDER, C. & HORSTMANN, G. (1977). Oral embryotoxicity study of Acesulfame K in rabbit, stock Hoe:HIMK (SPF Wiga) Report No. 317/77. Unpublished report. Submitted to WHO by Hoechst A.G.

BRUNK, R., WEIGAND, W., MAYER, D. & LANGER, K.H. (1986). Repeated-dose (2-week) oral toxicity study of acetoacetamide-N- sulfonic acid sodium salt in beagle dogs. Unpublished report No.86.0115. Submitted to WHO by Hoechst A.G.

CHRIST, O. & RUPP, W. (1976). Human experiments with Acetosulfam-^{14}C. Pharmacokinetics after oral administration of 30 mg to three healthy male probands. Unpublished report No.01-L42-0176-76. Submitted to WHO by Hoechst A.G.

CLAUSS (1981). Model experiments aimed at detecting possible reactions of Acesulfame K with constituents of food. Unpublished report. Submitted to WHO by Hoechst A.G.

DONAUBAUEER, H.H., MAYER, D. & LANGER, K.H.(1987). Antigenicity study of Acesulfame (H 73 3293) in guinea pigs. Active systemic anaphylaxis. Unpublished report No.87.1400. Submitted to WHO by Hoechst A.G.

ECKERT, H.G., DAGROSA, E. & RUPP, W. (1980). Tests with acetoacetamide-^{13}C,^{14}C in human subjects. Pharmacokinetics after oral administration of 50 mg to 3 healthy male volunteers. Unpublished report No.01-L42-0326-80. Submitted to WHO by Hoechst A.G.

ECKERT, H.G. & KELLNER, H. M. (1979). Kinetics of Acetoacetamide- (1-^{14}C) after single and repeated administration to rats. Unpublished report No.01-L42-0285-79. Submitted to WHO by Hoechst A.G.

EISENBRAND, G. (1979). Experiments aimed at the nitrosation of Acetosulfam. Unpublished report. Submitted to WHO by Hoechst A.G.

EISENBRAND, G. (1982). The nitrosatability of Acesulfame potassium in the nitrosamine assay procedure of WHO. Unpublished report. Submitted to WHO by Hoechst A.G.

ENGELBART, K. (1979). Ames test ß-hydroxybutyramid Substanz 90/79. Bericht Nr. 390/70A. Unpublished report. Submitted to WHO by Hoechst A.G.

FUCHS, H., HOLLANDER, H., MAYER, D. & LANGER, K.H. (1986). Repeated-dose oral toxicity study (3 months in food) of acetoacetamide-N-sulphonic acid in rats. Unpublished report No.86.0869. Submitted to WHO by Hoechst A.G.

GERICKE, D. (1977). Test for mutagenicity in bacteria strains in the absence and presence of a liver preparation. Unpublished report. Submitted to WHO by Hoechst A.G.

GROSS, G., GANTZ, KELLNER, H.-M. & ECKERT, H. (1987). Kinetics and metabolism of sodium-(1-^{14}C)-acetoacetamide-N-sulfonate after single doses in rats. Unpublished report No.01-L42-0495-87. Submitted to WHO by Hoechst A.G.

HOLLANDER, H. & WEIGAND, W. (1986). Sunett. Investigation of the inhalation toxicity of thermal degradation products in male and female SPF-Wistar rats. Unpublished Report No. 86.1398. Submitted to WHO by Hoechst A.G.

HOLLANDER, H., WEIGAND, W. & KRAMER, M. (1981). Toxicological investigation of acetoacetamide in a 90-day drinking water study with SPF-Albino Himalayan rabbits. Unpublished report No.0357/81. Submitted to WHO by Hoechst A.G.

HOLLANDER, H., WEIGAND, W. & KRAMER, M. (1985). Acetoacetamide-N- sulphonic acid, Na-salt. Subacute oral toxicity (28-day study) in SPF Wistar rats. Unpublished report No.85.0470. Submitted to WHO by Hoechst A.G.

JUNG, R. & HOLLANDER, W. (1986). Sunett. Study of the mutagenic potential in strains of *Salmonella typhimurium* (Ames test) and *Escherichia coli.* Unpublished Report No.86.0811. Submitted to WHO by Hoechst A.G.

KELLNER, H. M. & CHRIST, O. (1975a). Re: Absorption, distribution and elimination after administration of Acetosulfam-^{14}C to rats and dogs. Dr. Kn/tr 5547. Unpublished report. Submitted to WHO by Hoechst A.G.

KELLNER, H. M. & CHRIST, O. (1975b). Pharmacokinetic studies of Acetosulfam-^{14}C after oral administration to pigs. Unpublished report. Submitted to WHO by Hoechst A.G.

KELLNER, H. M. & ECKERT, H. (1983a). Re: Acesulfame K. Investigations of distribution in pregnant rats. Unpublished report No.01-L42-0379-83. Submitted to WHO by Hoechst A.G.

KELLNER, H. M. & ECKERT, H. (1983b). Re: Acesulfame K. Investigation into elimination with the milk. Unpublished report No.01-L42-0398-83. Submitted to WHO by Hoechst A.G.

KOETER, H.B.W.M. (1975). Effect of Hoe 0-95K on pregnancy of the rat. Report No. R4854. Unpublished report of CIVO TNO, Zeist. Submitted to WHO by Hoechst A.G.

KREILING, R. & JUNG, R. (1988a). Test for primary dermal irritation in the rabbit. Unpublished report No.88.1474. Submitted to WHO by Hoechst A.G.

KREILING, R. & JUNG, R. (1988b). Test for primary eye irritation in the rabbit. Unpublished report No.88.1564. Submitted to WHO by Hoechst A.G.

MARKERT, U. & WEIGAND, W. (1979a). Akute Toxizitat von Acesulfam-K am Zebrabarbling *(Brachydanio rerio)* Bericht Nr. 754/79. Unpublished report. Submitted to WHO by Hoechst A.G.

MARKERT, U. & WEIGAND, W. (1979b). Akute Toxizitat von Acesulfam-K an Goldorfen *(Leuciscus Idus f. melanotus)* Bericht Nr. 755/79. Unpublished report. Submitted to WHO by Hoechst A.G.

MARKERT, M. & JUNG, R. (1988). Sunett. 96-hour acute toxicity study in Zebra fish *(Brachydanio rerio).* Unpublished report No.881759. Submitted to WHO by Hoechst A.G.

MARQUARDT, H.W.J. (1978). Mutagenicity and oncogenicity of Acetosulfam-K. Studies with mammalian cells *in vitro.* Unpublished report of the Memorial Sloan-Kettering Cancer Centre, N.Y. Submitted to WHO by Hoechst A.G.

MAYER, D. & KRAMER, F. (1980). Studies on the effect of Acesulfame K on the Streptozotocin-diabetes in rats. Report No.464/80. Unpublished report. Submitted to WHO by Hoechst AG.

MAYER, D. & WEIGAND W. (1977). Acute intraperitoneal toxicity of Acesulfame-K, batch CIVO 4, in female SPF Wistar rats. Report No. 285/77. Unpublished report prepared for Hoechst A.G. Submitted to WHO by Hoechst A.G.

MAYER, D. & WEIGAND, W. (1985). Acetoacetamide-N-sulfonic, sodium salt. Micronucleus test in male and female NMRI mice after oral administration. Unpublished report No.85.0501. Submitted to WHO by Hoechst A.G.

MAYER, D., WEIGAND, W. & KRAMER, M. (1978a). Acesulfame K Cytogenetic study in the Chinese hamster. Report No. 575/78. Unpublished report. Submitted to WHO by Hoechst A.G.

MAYER, D., WEIGAND, W. & KRAMER, M. (1978b). Report on investigations of the caecum-enlarging action of Acesulfam-K salt in juvenile female rats. Report No. 416/78. Unpublished report. Submitted to WHO by Hoechst A.G.

MAYER, D., WEIGAND, W. & KRAMER, M. (1978c). A report on the experimental studies of the effect of Acesulfame K salt in causing caecal enlargement in adult female rats. Report No. 417/78. Unpublished report. Submitted to WHO by Hoechst A.G.

MAYER, D., WEIGAND, W. & KRAMER, M. (1978d). Report on studies of the subchronic effects on rats of potassium chloride in food. Report No.418/78. Unpublished report. Submitted to WHO by Hoechst A.G.

MAYER, D., WEIGAND, W. & KRAMER, M. (1978e). Repeated-dose (one month) oral toxicity study of acetoacetamide administered to SPF Wistar rats in the feed (Range-finding study). Report No. 603/78. Unpublished report. Submitted to WHO by Hoechst AG.

MAYER, D., WEIGAND, W. & KRAMER, M. (1979). Repeated-dose (13-weeks) toxicity study of acetoacetamide administered in the feed to SPF Wistar rats in the feed. Report No. 691/79. Unpublished report. Submitted to WHO by Hoechst AG.

MAYER, D., WEIGAND, W. & KRAMER, M. (1980). Tolerance study with acetoacetamide. 14-times oral administration to male and female beagle dogs. Unpublished report No. 175/80. Submitted to WHO by Hoechst A.G.

MÜLLER, W. (1988a). Evaluation of Acetoacetamide-N-sulfonic in the unscheduled DNA synthesis test in mammalian cells *in vitro.* Unpublished report No.88.0665. Submitted to WHO by Hoechst A.G.

MÜLLER, W. (1988b). Acetoacetamide-N-sulfonic, sodium salt. Detection of gene mutations in somatic cells in culture. HGPRT-test with V 79 cells. Unpublished report No.88.1452. Submitted to WHO by Hoechst A.G.

MÜLLER, W. (1988c). Acetoacetamide-N-sulfonic, sodium salt. Study of the mutagenic potential in strains of *Salmonella typhimurium* (Ames test) and *Escherichia coli.* Unpublished report No.88.1442. Submitted to WHO by Hoechst A.G.

MÜLLER, W. (1989a). Acetoacetamide. Detection of gene mutations in somatic cells in culture. HGPRT-test with V79 cells. Unpublished report No.89.1152. Submitted to WHO by Hoechst A.G.

MÜLLER, W. (1989b). Acetoacetamide. Chromosome aberrations *in vitro* in V79 Chinese hamster cells. Unpublished report No.89.0996. Submitted to WHO by Hoechst A.G.

MÜLLER, W. (1989c). Evaluation of acetoacetamide in the unscheduled DNA synthesis test in mammalian cells *in vitro.* Unpublished report No.89.1021. Submitted to WHO by Hoechst A.G.

MÜLLER, W. (1989d). Acetoacetamide-N-sulfonic. Chromosome aberrations *in vitro* in V 79 Chinese hamster cells. Unpublished report No.89.0046. Submitted to WHO by Hoechst A.G.

MYHR, B.C. & BRUSICK, D.J. (1982). Evaluation of Acesulfame K "CIVO-4" in the primary rat hepatocyte unscheduled DNA synthesis assay. Unpublished report of Litton Bionetics No.21001. Submitted to WHO by Hoechst A.G.

NEWMAN, A.J. (1982). Pathology report of the combined chronic toxicity and carcinogenicity study with Acesulfame K in rats. Unpublished report. Submitted to WHO by Hoechst A.G.

READ, M.H., MOYSAN, F., PETRA, D., RADA, G., LAGARDE, V. & ATTIA, M. (1989a). Acetoacetamide-N-sulfonic acid. Toxicity study by repeated oral administration for 13 weeks in Cynomolgus monkeys. Unpublished report, Study No. 3915TCP of the Centre International de Toxicologie. Submitted to WHO by Hoechst A.G.

READ, M.H., PETRA, D., RADA, G. & ATTIA, M. (1989b). Acetoacetamide-N-sulfonic, sodium salt. Toxicity study by repeated oral administration for 30 days in Cynomolgus monkeys. Unpublished report, Study No. 4393 TSP, of the Centre International de Toxicologie. Submitted to WHO by Hoechst A.G.

REUZEL, P.G.J. & VAN DER HEIJDEN, C.A. (1977). Long-term (two-year) oral toxicity study with Hoe 0-95K in beagle dogs. Report No. 5280. Unpublished report of CIVO TNO Zeist. Submitted to WHO by Hoechst A.G.

ROHRBORN, G. (1976). Expert opinion on the mutagenicity of Acetosulfame K. Unpublished report of Universität Düsseldorf. Submitted to WHO by Hoechst A.G.

ROSENKRANZ, B., MALERCZYK, V. & ROSENBERGER, S. (1988). Safety and tolerability of Acetoacetamide-N-sulfonic acid (H 84 7026) administered to healthy male volunteers. Unpublished report No.Hoe 095 K/I/D/101/-. Submitted to WHO by Hoechst A.G.

RUPPRICH, N. & WEIGAND, W. (1984a). Acetoacetamide-N-sulfonic acid sodium salt. Test of the acute oral toxicity in male and female Wistar rats. Unpublished report No.84.0421. Submitted to WHO by Hoechst A.G.

RUPPRICH, N. & WEIGAND, W. (1984b). Acetoacetamide-N-sulfonic acid sodium salt. Test of the acute intravenous toxicity in male and female Wistar rats. Unpublished report No.84.0422. Submitted to WHO by Hoechst A.G.

SAGELSDORFF, P., LUTZ, W.K. & SCHLATTER, Ch. (1981). Research Report No. 40182A. Unpublished report. Submitted to WHO by Hoechst A.G.

SCHOLTHOLT, J. & ALPERMANN, H.G. (1984). Pharmacological investigations on acetoacetamide-N-sulfonic acid sodium salt. Unpublished report. Submitted to WHO by Hoechst A.G.

SCHRINNER & LIMBERT (1977). Report on investigations of the action of the sweetener Acesulfame K against bacteria. Unpublished report. Submitted to WHO by Hoechst A.G.

SINKELDAM, E.J. (1976). Reproduction study with Hoe-0-95K in rats. Report No. R5187. Unpublished report of CIVO TNO, Zeist. Submitted to WHO by Hoechst A.G.

SINKELDAM, E.J. ET AL. (1976). Multigeneration study with Hoe 0-95K in rats. Report No. 5156. Unpublished report of CIVO TNO, Zeist. Submitted to WHO by Hoechst A.G.

SINKELDAM, E.J., KUPER, C.F. & BEEMS, R.B. (1979). Combined chronic toxicity and carcinogenicity study with Hoe O-95K in rats pretreated *in utero.* Report No. R6100. Unpublished report of CIVO TNO, Zeist. Submitted to WHO by Hoechst A.G.

SINKELDAM, E.J., TIL, H.P. & WILLEMS, M.I. (1974). Sub-chronic (90-day) toxicity study with Hoe 0-95K in albino rats. Unpublished report of CIVO TNO, Zeist. Submitted to WHO by Hoechst A.G.

SINKELDAM, E.J., van der HEIJDEN, C.A. & KOETER, H.B.W.M. (1977). Combined chronic toxicity and carcinogenicity study with Hoe 0-95K in rats. Report No. R5229. Unpublished report of CIVO TNO, Zeist. Submitted to WHO by Hoechst A.G.

VOGEL, H.P. & ALPERMANN, H.G. (1974). Sweetener O-95K (H 73 3293). Pharmacological investigations. Unpublished report. Submitted to WHO by Hoechst A.G.

VOLZ, M. (1975). Unpublished Research Report RCL Dr.Vz/Tr No.01- L42-0143-75. Submitted to WHO by Hoechst A.G.

VOLZ, M. (1976). Unpublished Research Report No. 01-L42-0177-76. Submitted to WHO by Hoechst A.G.

VOLZ, M. & ECKERT, H. (1981). Unpublished Research Report No. 01-142-0352-81. Submitted to WHO by Hoechst A.G.

VOLZ, M., ECKERT, H. & KELLNER, H.M. (1983). Investigations into the kinetics and biotransformation of the sweetener Acesulfame K following repeated high-dose administration. Unpublished Research Report No. 01-L42-0396-83. Submitted to WHO by Hoechst A.G.

VOLZ, M. & FEHLHABER, H.W. (1981). Studies on the metabolism of acetoacetamide-^{14}C in rats, dogs, hamsters, rabbits and humans. Unpublished report No.01-L42-0336-81. Submitted to WHO by Hoechst A.G.

WILLEMS, M.I. (1974). Dominant lethal assay with Hoe 0-95K in male albino rats. Report No. R4472. Unpublished report of CIVO, TNO. Submitted to WHO by Hoechst A.G.