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MUT/07/23

COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

STUDY ON THE MUTAGENICITY OF SODIUM BENZOATE AND POTASSIUM SORBATE

Introduction

1. Sodium benzoate (E211) and potassium sorbate (E202) are two examples of organic acid food preservatives based on benzoic and sorbic acids. Benzoic acid and its sodium, potassium and calcium salts and sorbic acid and its potassium and calcium salts are permitted for use in a wide range of foods in the EU which is transcribed into UK law under the Miscellaneous Food Additive Regulations 1995. These regulations lay down maximum levels for the use of these preservatives in specific foods so that the acceptable daily intake (ADI) is not exceeded by the majority of the population.

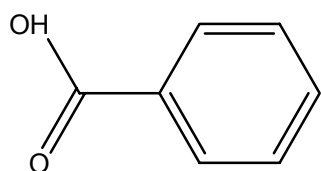


Figure 1: Benzoic acid

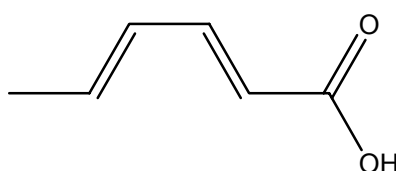


Figure 2: Sorbic acid

2. In 1999, a study was published in *Free Radical Biology and Medicine* by Professor Peter Piper then from University College London which raised the possibility that these preservatives may be mutagenic to the yeast mitochondrial genome (Piper, 1999).

3. This study used genetically modified yeast cells in an *in vitro* system to demonstrate the effects of potassium sorbate and sodium benzoate on the respiratory capabilities of the cells. Yeast superoxide dismutase mutant *S. cerevisiae* cells were incubated with the two preservatives and the effects observed using a halo assay. The author concluded that the test substances produced an increased number of respiratory-deficient yeast cells under aerobic conditions which indicates that damage was occurring to the mitochondrial DNA in the yeast cells. This paper has attracted considerable interest and COM is invited to comment on the implications of the results for the safety of these preservatives.

4. The published paper by Professor Piper from 1999 can be found as Annex 1.

Sorbic acid

5. Sorbic acid has been assessed for safety by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1974).

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6. There are a number of genotoxicity studies, both *in vitro* and *in vivo*, looking at sorbic acid and its salts. A brief outline of these studies can be found in table 1. Annex 2 contains a summary of the other toxicity studies relating to these compounds and considered by JECFA and a literature review of studies published since the JECFA opinions were finalised. No genotoxicity studies were available for the JECFA assessment.

7. Carcinogenicity studies on sorbic acid showed no links between sorbic acid and an increased risk of tumours. Further details of these carcinogenicity studies can be found in annex 2.

End Point	Test Object	Concentration or dose	Result	Reference
In Vitro studies				
Reverse mutation	<i>S. typhimurium</i> TA98, TA100	≤ 2mg/plate PS	Negative (+/- S9)	Munzner <i>et al</i> , 1990
Reverse mutation	<i>S. typhimurium</i> TA100, TA1535	≤ 5mg/plate SA	Negative (+/- S9)	Jung <i>et al</i> , 1992
Reverse mutation	<i>S. typhimurium</i> TA92, TA1535, TA100, TA1537, TA94, TA98	≤ 3mg/plate PS, ≤ 10mg/plate SA	Negative (PS & SA)	Ishidate <i>et al</i> , 1984
Reverse mutation	<i>S. typhimurium</i> TA98 and TA100	Urine from Jcl-ICR mice dosed with sorbic acid at 22500 mg/kg bw/day and potassium sorbate at 2010, 10050 & 30150 mg/kg bw/day for 12 months.	Negative (SA & PS)	Tsuchiya & Yamaha, 1983
Reverse mutation	<i>S. typhimurium</i> TA98	Extracts of gut contents from Jcl-ICR mice dosed with sorbic acid at 22500 mg/kg bw/day and potassium sorbate at 2010, 10050 & 30150 mg/kg bw/day for 12 months.	Negative (PS 2010 & 10050 mg/kg bw/day); Equivocal (PS 30150 mg/kg bw/day) Positive (SA)	Tsuchiya & Yamaha, 1984
Reverse mutation	<i>S. typhimurium</i> TA98 and TA100	0.003, 0.03, 3, 30 & 300 µg/plate SA-methylamine reaction products	Negative (+/- S9)	Ferrand <i>et al</i> , 2000a
Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA102, TA1535, TA1537	10, 1, 0.1, 10 ⁻² & 10 ⁻³ mg/ml reaction products of SA with one of : methylamine, ethylamine, propylamine, butylamine, benzylamine	Negative for all reaction products (+/- S9)	Ferrand <i>et al</i> , 2000b
Mammalian	V79 hamster	≤0.5% PS	Negative	Budayova,

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gene (forward) mutation	cells			1985
Mammalian gene (forward) mutation	Chinese hamster ovary cells	≤ 20mg/ml PS, ≤1mg/ml SS (stored & fresh solutions of both)	Negative (+/- S9)	Munzner <i>et al</i> , 1990
Mammalian gene (forward) mutation	Chinese hamster V79 cells	≤ 1.05 mg/ml SA ≤ 20 mg/ml PS ≤ 0.8 mg/ml SS	Negative (SA & PS) Positive (SS, all concs)	Hasegawa <i>et al</i> , 1984
Chromosomal aberration	Chinese hamster fibroblast cells	≤ 4 mg/ml PS ≤ 1 mg/ml SA	Positive (PS) Negative (SA)	Ishidate <i>et al</i> , 1984
Chromosomal aberration	Chinese hamster fibroblast cells	≤ 4 mg/ml PS	Weakly positive	Ishidate & Odashima, 1977
Chromosomal aberration	Chinese hamster V79 cells	≤ 1.05 mg/ml SA ≤ 20 mg/ml PS ≤ 0.8 mg/ml SS	Negative (SA ≤ 0.7mg/ml, PS ≤ 10mg/ml, SS = 0.2 mg/ml). Positive at higher concentrations	Hasegawa <i>et al</i> , 1984
Chromosomal aberration	Chinese hamster cells	≤ 6.01mg/ml PS	Positive (≥ 3mg/ml)	Abe & Sasaki, 1977
Micronucleus assay	Syrian Hamster Embryo Fibroblasts	120, 300, 600 and 1200 µg/ml SA (freshly prepared) and SS (freshly prepared or heated, sonicated and stored) and PS (freshly prepared)	Negative (freshly prepared SA, SS & PS) Positive (heated, sonicated and stored SS)	Schiffmann & Schlatter, 1992
Sister chromatid exchange	Chinese hamster ovary cells	≤ 20mg/ml PS, ≤1mg/ml SS (stored & fresh solutions of both)	Negative (+/- S9)	Munzner <i>et al</i> , 1990
Sister chromatid exchange	Chinese hamster V79 cells	≤ 1.05 mg/ml SA ≤ 20 mg/ml PS ≤ 0.8 mg/ml SS	Negative (SA ≤ 0.7mg/ml, PS ≤ 5mg/ml, SS = 0.2 mg/ml). Positive at higher concentrations.	Hasegawa <i>et al</i> , 1984
Sister chromatid exchange	Chinese hamster cells	≤ 6.01mg/ml PS	Negative	Abe & Sasaki, 1977
Unscheduled DNA synthesis	Human type II lung tumour cells	≤ 2 mg/ml SA	Negative	Jung <i>et al</i> , 1992
Alkaline elution assay	Human type II lung tumour cells	≤ 1 mg/kg bw PS	Negative	Jung <i>et al</i> , 1992
DNA repair assay	HeLa cells and DNA plasmids from <i>E. coli</i>	10, 1, 0.1, 10 ⁻² & 10 ⁻³ mg/ml SA-methylamine reaction products	Negative	Ferrand <i>et al</i> , 2000a
DNA repair assay	HeLa cells and DNA plasmids from <i>E. coli</i>	10, 1, 0.1, 10 ⁻² & 10 ⁻³ mg/ml reaction products of SA with one of :	Negative for all reaction products	Ferrand <i>et al</i> , 2000b

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		methylamine, ethylamine, propylamine, butylamine, benzylamine		
Transformation assay	Syrian hamster embryos	120, 300, 600 & 1200 µg/ml SA, PS & SS	Negative (All SA & PS & freshly prepared SS) Positive (heated, sonicated & stored SS)	Schiffmann & Schlatter, 1992
In Vivo Studies				
Micronucleus assay	Mouse bone marrow	100 and 200 mg/kg bw/day PS or SS. (IP or gavage)	Negative (PS fresh and stored and SS fresh); positive (SS stored given by IP and gavage)	Munzner <i>et al</i> , 1990
Micronucleus assay	Chinese hamster bone marrow	100 and 200 mg/kg bw/day PS or SS. (IP or gavage)	Negative (PS fresh and stored and SS fresh); positive (SS stored by IP but negative by gavage)	Munzner <i>et al</i> , 1990
Micronucleus assay	Mouse bone marrow	500, 1500 or 5000 mg/kg bw SA	Negative	Jung <i>et al</i> , 1992
Chromosomal aberration	Mouse bone marrow	15mg/kg bw/day (SA only), 7.5mg/kg bw/day (SA with 1mg/kg bw/day sodium nitrate)	Negative (SA only) Positive (SA and sodium nitrate)	Banerjee & Giri, 1986
Spindle abnormalities	Mouse bone marrow	15mg/kg bw/day (SA only), 7.5mg/kg bw/day (SA with 1mg/kg bw/day sodium nitrate)	Positive (SA only and SA with sodium nitrate)	Banerjee & Giri, 1986
Sister chromatid exchange	Chinese hamster bone marrow	100 and 200 mg/kg bw/day PS or SS. (IP or gavage)	Negative (both substances and routes of administration)	Munzner <i>et al</i> , 1990
Sister chromatid exchange	Mouse bone marrow	500, 1500 or 5000 mg/kg bw SA	Negative	Jung <i>et al</i> , 1992
Alkaline elution assay	Rat liver cells	400, 800, 1200 mg/kg bw PS	Negative	Jung <i>et al</i> , 1992
Somatic mutation assay	<i>D. melanogaster</i>	0.4, 0.6, 0.8, 1.25, 2.5 & 3.35 mg/ml SS, 2.5 & 3.75 mg/ml PS	Negative (but some toxic effects seen in the larvae)	Schlatter <i>et al</i> , 1992

Table 1: Results of genotoxicity studies using sorbic acid and its salts. (SA = sorbic acid, PS = potassium sorbate, SS= sodium sorbate)

Mechanistic studies

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8. Jcl/ICR mice were fed a diet containing 15% sorbic acid (equivalent to 22500mg/kg bw/day) for 12 weeks. A 2-fold induction of peroxisome β -oxidation, 1.9-fold induction of urate oxidase and 1.7-fold induction of catalase were seen in liver homogenates. No changes were observed in mitochondrial β -oxidation. A 3.6-fold induction of 2,4-dienoyl-CoA hydratase towards sorboyl CoA and a 1.3-fold induction of 2,4-dienoyl-CoA reductase, leading to a 3-fold elevation in the hydratase : reductase ratio. Cellular glutathione levels were depleted to 40% of controls by the end of the study. The authors concluded that oxidative stress caused by depleted cellular glutathione pool and peroxisome proliferation may indicate a non-mutagenic link between sorbic acid and hepatocellular carcinoma (Nishimaki-Mogami *et al*, 1991).

9. V79 Chinese hamster ovary cells and EUE human fibroblasts were used to assess the effects of sodium nitrate and potassium sorbate on cell growth, proliferation and damage. An unspecified volume of 1% potassium sorbate solution was associated with significantly reduced cell proliferation and DNA synthesis in V79 cells. Protein synthesis in V79 cells showed a concentration-dependent reduction. Potassium sorbate concentrations less than 0.5% showed no effects on DNA synthesis, but levels of 0.5% and 1% produced a temporary reduction in the rate of DNA synthesis followed 180 minutes later by a rapid increase in DNA synthesis (Budayova, 1985).

10. V79 cells were exposed to sodium sorbate at concentrations of 0.4, 0.6, 0.8, 1.25, 2.5 mg/ml and potassium sorbate at 2.5 mg/ml. Solutions were either used fresh or stored for 3, 28 or 208 days. Sodium chloride was used as a high salt control at 2.5 mg/ml. At 2.5 mg/ml sodium sorbate, the number of viable cells decreased 26% with an 18hr recovery period and 40% without the recovery period. Intracellular vacuoles were detectable following treatment and this correlated with a concentration-dependent increase in the G₂/M phase cells, a decrease in S-phase cells and an increase in the cellular protein content in both the G₁ and G₂ phase cells. At lower levels, no effects were noted. Sodium chloride produced a similar reduction in cell viability, but this was less marked than with the sodium sorbate. Following storage, the sorbate solutions produced a significant increase in cells with cell-cycle arrest compared to controls and fresh solutions of sorbates, particularly when no recovery period was allowed. Potassium sorbate produced similar results to sodium sorbate except that the stored solution produced a greater effect on the cell-cycle.

11. The authors concluded that sodium and potassium sorbate have a weak aneugenic activity and that the effects at high concentrations may be due to osmotic pressure of the culture medium. The authors conclude that the cytotoxic effects observed were attributable to the occurrence of 4,5-epoxy-2-hexenoic acid in sorbic acid solutions following storage (Schlatter *et al.*, 1992).

12. Hepatocytes from male Wistar rats induced with phenobarbitol were exposed to potassium sorbate at concentrations of 1, 2, 5 and 10 mM. Experiments were carried out with normal hepatocytes and hepatocytes that

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had been incubated in medium containing a linolenic acid-bovine serum albumin complex (LNA-loaded cells) in order to assess the extent of lipid peroxidation occurring in these cells. Following incubation the medium was assayed for malondialdehyde (MDA) and lactic acid dehydrogenase (LDH). In normal hepatocytes, no effects were seen with potassium sorbate at any concentration except a reduction in glutathione levels was noted at concentrations above 1mM which was more pronounced in the LNA-loaded cells. In LNA-loaded cells, 5mM potassium sorbate produced a slight but significant increase in MDA accumulation which increased to 1.8nmol/10h/mg protein at 10mM. A 20.8% increase in LDH release was seen following incubation of LNA-loaded cells with potassium sorbate at 10mM. The presence of an antioxidant, N,N'-diphenyl-p-phenylenediamine (DPPD) appeared to reduce the effects produced by potassium sorbate (Sugihara et al., 1997).

13. Cultured rat hepatocytes exposed to potassium sorbate for 10 hours at concentrations of 1-10mM showed a significant reduction in cellular glutathione which returned to normal after 6 hours at 1.0 and 2.5mM and within 36 hours at 5mM. With potassium sorbate alone there was no increase in malondialdehyde (MDA), but LDH leakage was increased at 10mM.

14. Following addition of eicosapentaenoic acid (EPA) to concentrations of potassium sorbate, lipid peroxidation was enhanced parallel to the reduction in glutathione. Addition of antioxidants almost completely prevented the lipid peroxidation observed in cells exposed to EPA and potassium sorbate. The authors concluded that cells exposed to potassium sorbate may be more susceptible to oxidative stress (Sugihara *et al*, 1998).

Benzoic Acid

15. Benzoic acid and its salts have been assessed for safety by JECFA (JECFA, 1996). These compounds were assessed alongside benzyl acetate, benzyl alcohol and benzaldehyde as these compounds are all metabolised to benzoic acid which is further metabolised to hippuric acid and excreted. This paper focuses on benzoic acid and its salts.

16. There are a number of genotoxicity studies, both *in vitro* and *in vivo*, looking at benzoic acid and its salts. A brief outline of these studies can be found in table 2. Annex 2 contains a summary of the other toxicity studies relating to these compounds and considered by JECFA and a literature review of studies published since the JECFA opinions were finalised. Since the JECFA assessment, no new carcinogenicity studies on benzoic acid or its salts have been carried out.

End Point	Test Object	Concentration	Result	Reference
In Vitro studies				
Reverse mutation	<i>S. typhimurium</i> TA92, TA94, TA98, TA1535, TA1537	≤ 10000 µg/plate (BA) ≤ 3000 µg/plate (SB)	Negative	JECFA, 1996
Reverse	<i>S. typhimurium</i>	33-10000	Negative	JECFA,

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mutation	TA97, TA100, TA1537	TA98, TA1535,	µg/plate (substance not specified)		1996
Reverse mutation	<i>S. typhimurium</i> TA92, TA100, TA94, TA98	TA1535, TA1537,	≤ 3 mg/plate SB, ≤ 10 mg/plate BA	Negative (BA & SB)	Ishidate <i>et al</i> , 1984
Chromosomal aberration	Chinese hamster lung cells		≤ 1500 µg/ml (BA); ≤ 2000 µg/ml (SB)	Equivocal (BA); positive (SB)	JECFA, 1996
Chromosomal aberration	Chinese hamster cells		≤ 1.4 mg/ml SB	Positive	Abe & Sasaki, 1977
Chromosomal aberration	Chinese hamster fibroblast cells		≤ 2 mg/ml SB	Positive	Ishidate & Odashima, 1977
Chromosomal aberration	Chinese hamster fibroblast cells		≤ 2 mg/ml SB ≤ 1.5 mg/ml BA	Equivocal – BA, Positive SB	Ishidate <i>et al</i> , 1984
Sister chromatid exchange	Chinese hamster ovary cells		≤ 1220 mg/ml (substance not specified)	Equivocal	JECFA, 1996
Sister chromatid exchange	Human lymphoblastoid cells		≤ 3660 mg/ml (substance not specified)	Negative	JECFA, 1996
Sister chromatid exchange	Human lymphocytes		≤ 244 mg/ml (substance not specified)	Negative	JECFA, 1996
Sister chromatid exchange	Chinese hamster cells		≤ 1.4 mg/ml SB	Negative	Abe & Sasaki, 1977

Table 2: Results of genotoxicity studies using benzoic acid and its salts. (BA = benzoic acid, SB = sodium benzoate)

Mechanistic Studies

17. Hepatocytes from male Wistar rats induced with phenobarbitol were exposed to sodium benzoate at concentrations of 1, 2, 5 and 10 mM. Experiments were carried out with normal hepatocytes and hepatocytes that had been incubated in medium containing a linolenic acid-bovine serum albumin complex (LNA-loaded cells) in order to assess the extent of lipid peroxidation occurring in these cells. Following incubation the medium was assayed for malondialdehyde (MDA) and lactic acid dehydrogenase (LDH). In normal hepatocytes, no effects were seen with sodium benzoate at any concentration although a reduction in glutathione was noted at concentrations above 1mM which was more pronounced in the LNA-loaded cells. No adverse effects were seen on the LNA-loaded cells when exposed to sodium benzoate although a slight reduction in glutathione level was observed when cells were exposed to concentrations above 5mM. No effects on extracellular LDH were seen at any concentration following incubation of these cells with sodium benzoate (Sugihara et al., 1997).

Summary

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18. Sorbic and benzoic acids and their salts are approved as food additives within the European Union. JECFA have previously assessed these additives for safety and have concluded that their use in foods poses no appreciable risk to health at levels up to 25 mg/kg bw/day for sorbic acid and its salts and 5 mg/kg bw/day for benzoic acid and its salts. A number of studies have been published since the JECFA assessments. The majority of these studies show no indication that sorbic or benzoic acids or their salts are associated with adverse health effects. Genotoxicity and mechanistic studies for both groups of compounds gave primarily negative results and long term carcinogenicity and reproduction studies showed no adverse health effects. Some of the studies published since the JECFA evaluation used very high dose levels (~15% in the diet) which can cause nutritional imbalance and may not be relevant to lower intakes of these additives.

19. A study published in 1999 by Professor P. Piper used modified yeast cells to demonstrate the effects of potassium sorbate and sodium benzoate on the respiratory capabilities of the cells. The author concluded that the test substances produced a significant number of respiratory-deficient yeast cells which indicates that damage is occurring to the mitochondrial DNA in the yeast cells.

Questions for the Committee

1. Members are requested to comment on the relevance for human health of the findings in Professor Piper's paper.
2. On the basis of the available data can members advise on whether sorbic and benzoic acids and their salts have mutagenic properties?

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Annex 1

COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

STUDY ON THE MUTAGENICITY OF SODIUM BENZOATE AND POTASSIUM SORBATE

Piper P. (1999) Yeast superoxide dismutase mutants reveal a pro-oxidant action of weak organic acid food preservatives. *Free Radical Biology and Medicine*. 27 (11/12) 1219-1227.

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Annex 2

COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

STUDY ON THE MUTAGENICITY OF SODIUM BENZOATE AND POTASSIUM SORBATE

JECFA Considerations

Summaries of the genotoxicity studies looked at by JECFA can be found in tables 1 and 2, starting on page 2 in the main paper.

Sorbic acid and its salts

Metabolism

1. *In vitro* studies using microsomes from rat liver show that sorbic acid is oxidised via 2-carbon atom fragments which recondense to form acetoacetate.
2. Around 85% of a dose of radio-labelled sorbic acid (species and route of exposure not stated) was excreted through the expired air as carbon dioxide. Of the dose that wasn't excreted prior to sacrifice, 3% was found in the skeletal muscles and 6.6% in the other parts of the carcass; the majority of which was found in the subcutaneous fat deposits and in organ lipids (JECFA, 1974).

Short term studies

3. Acute toxicity studies in rats and mice gave LD50 values of between 1300 mg/kg bw and 10500 mg/kg bw for sorbic acid and its potassium and sodium salts. Short term feeding studies were available in rats, rabbits and dogs and gavage studies were available in mice. Adverse effects noted included decreased growth rates in mice at doses of 80mg/kg when administered for 3 months. Short term studies in rats administered doses equivalent to 500 -1000 mg/kg bw/day sorbic acid in the diet caused slight enlargement of the liver compared to controls. This finding was confirmed in several studies with higher doses of sorbic acid. Doses equivalent to 5000 mg/kg bw/day administered in the diet caused raised blood cholesterol and increased fat deposition in the internal organs, depression of leukocyte numbers and impairment of cholinesterase activity. Doses equivalent to 500-1000 mg/kg bw/day administered for 3 months in the diets of rabbits and dogs showed no adverse health effects.

Long-term studies

4. A long term feeding study in mice (duration not specified) given 40mg/kg bw/day sorbic acid in the diet produced no effects except reduction

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in liver, kidney and testes weights compared to controls. Several long term feeding studies in rats were available with doses of between 40mg/kg bw/day and 2500mg/kg bw/day and no effects were noted.

JECFA conclusions

5. The JECFA concluded that the rat was an extremely good model for the metabolism of sorbic acid and its salts, as man and the rat metabolise the free ions in the same way. The Committee identified a NOAEL of 2500mg/kg bw/day from the rat studies and derived an Acceptable Daily Intake (ADI) level for humans of 25 mg/kg bw/day (JECFA, 1974).

Benzoic acid and its salts

Metabolism

6. Benzoic acid is rapidly absorbed in many species. In rats fed a diet containing 1.5% benzoic acid, 95% of the dose was excreted in the urine as hippuric acid. When the dose was increased to 3.75% of the diet, the ratio of hippuric acid to total benzoic acid decreased, but additional glycine in the diet increased elimination to 86-99%. In man, the liver is the main organ involved in metabolism of benzoic acid and its salts, where conjugation with glycine occurs followed by rapid excretion via the urine. In man, following high doses of benzoic acid, up to 3% of the dose was conjugated with glucuronic acid and all metabolites were eliminated completely within 14 hours. Up to 75-80% of an administered dose of benzoic acid was eliminated within 6 hours and through measuring metabolites in the urine, it was shown that a significant proportion of the dose was excreted in the urine as hippuric acid (JECFA, 1996).

Short term studies

7. Acute oral studies gave LD50 values for benzoic acid and its sodium salt of between 1714 and 2700 mg/kg bw.

8. Short term toxicity studies in rats given benzoic acid and its salts at doses of 16-4000 mg/kg bw/day showed no adverse effects at doses less than 750 mg/kg bw/day. Significant reductions in body weight and food consumption compared to controls were seen at doses of around 1000 mg/kg bw/day. Many deaths occurred at doses above 1500 mg/kg bw/day following hyper-excitability, urinary incontinence, incoordination, tremor and convulsions. In one study, kidney and liver weights were found to be higher in test animals that survived the treatment period when compared to controls.

9. Groups of 4 guinea pigs given a dose of 150 mg/kg bw/day benzoate plus benzoic acid for 65 days showed no adverse effects.

10. Seventeen dogs given 1000 mg/kg bw/day benzoate or benzoic acid showed no adverse effects when dosed for 250 days. Higher doses produced ataxia, convulsions and death (JECFA, 1996).

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Long-term toxicity and carcinogenicity

11. Groups of 50 mice of each sex were administered sodium benzoate at doses of 6200 mg/kg bw/day for males and 5960 mg/kg bw/day for females in their drinking water for their lifetime until death occurred or until sacrificed when moribund. Complete necropsy showed no treatment related effects and no effects were seen on body weights, survival or incidence of tumours in the tissues studied.

12. Benzoic acid was administered to rats in their diets for their lifetime at doses of 40-750 mg/kg bw/day. Doses of 500mg/kg bw/day showed no treatment related effects however, at doses above this, reduced survival rates, bodyweights and food intakes were observed in treatment groups compared to controls.

13. Sodium benzoate was administered to rats in their diets at doses of 500 and 1000 mg/kg bw/day for 78-104 weeks. No adverse treatment related effects were observed except statistically insignificant differences between bodyweights and mortality rates between treatment and control groups. A variety of tumours were found, but these were not considered to be treatment related (JECFA, 1996).

Developmental toxicity in rats

14. Sodium benzoate was administered by intraperitoneal injection at doses of 100, 315 or 1000 mg/kg bw/day on days 9-11 or 12-14 of gestation. Control animals were administered 90 or 100 mg/kg bw/day sodium chloride on the same days as the treatment groups. Foetal body weights were reduced in the high dose treated animals in both groups (9-11 days and 12-14 days). The incidences of deaths in utero were reported to be higher in the treatment groups compared to controls but no statistical analysis was provided. No gross abnormalities were seen in the treatment groups dosed on days 12-14 of gestation, but an increase was reported in the animals given 1000 mg/kg bw/day on days 9-11 of gestation but no statistical analysis was provided.

15. Sodium benzoate was administered by gavage to multiple species at the following doses:

Species	No. per dose group	Doses (mg/kg bw)	Treatment period	Time of necropsy
CD-1 mice	20	1.75, 8, 38, 175.	Days 6-15	Day 17
Wistar rats	24	1.75, 8, 38, 175	Days 6-15	Day 20
Golden Hamsters	20	3, 14, 65, 300	Days 6-10	Day 14
Dutch belted rabbits	10	2.5, 12, 54, 250	Days 6-18	Day 29

Table 1: Doses of sodium benzoate administered to different species and at different times.

16. A positive control group was included for each species with rats and mice receiving aspirin at 150 mg/kg bw/day, hamsters receiving aspirin at 250 mg/kg bw/day and rabbits receiving 2.5 mg/kg bw/day 6-aminonicotinamide. No observable adverse effects were seen, or differences in maternal body weight measured between control and test groups. Total number of

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pregnancies, maternal survival, body weight, total number of corpora lutea and implantation sites per pregnant female, total number of live litters, fetuses, and resorptions per litter, sex ratio, total number of dead fetuses, number of dams with one or more dead fetuses, average foetal weight and incidences of gross, skeletal and soft tissue abnormalities were all measured. No significant deviations from the control values and no dose-related effects were reported. Incidence of teratogenic observations was comparable between test and control groups.

17. Groups of 27-30 pregnant Wistar rats were provided with diets containing 0, 1, 2, 4 and 8% which were calculated to be equivalent to 0, 700, 1310, 1875 or 965 mg/kg bw/day sodium benzoate on days 1-20 of gestation. The sodium benzoate intake of the 8% diet group was significantly reduced due to reduced food consumption. The numbers of viable and dead fetuses, early and late resorptions, foetal, placental and ovarian weights were measured and abnormalities of maternal organs and foetal appearance were recorded. Skeletal and visceral abnormalities were investigated. Five dams from each group were allowed to deliver naturally and the number of offspring, survival, bodyweight and abnormalities were recorded. Three weeks after birth, pups were weaned, examined for gross abnormalities and half the pups were necropsied along with all the dams. Food consumption and body weights were measured in the remaining pups that were then necropsied at 8 weeks of age. The two lowest dose groups demonstrated no significant biological differences to controls. In the 4% group, dams did not gain weight and in the highest dose group, dams lost weight although no statistical analysis was carried out. Food intake was markedly reduced in the two higher dose groups. Also in these groups, the numbers of dead or resorbed fetuses were significantly increased and the average body weight of viable fetuses was significantly lower compared to controls. Mild systemic oedema, anophthalmia, pyelectasis, microphthalmia, hydrocephalus and cerebral hypoplasia were also observed. The incidence of delayed ossification, lumbar or cervical ribs and varied sternbrae were significantly increased in the 4% (96.5%) and 8% (100%) groups (% increase supplied but no actual figures). Of the pups that were delivered naturally, the two high dose groups demonstrated reduced delivery rates (50% and 8.2% delivery respectively) with complete loss of litters after parturition. The authors conclude that the effects in dams and fetuses in the two high dose groups were due to reduced maternal food intake leading to malnutrition rather than dose related as the intake of sodium benzoate in the 8% group was actually lower than the 2% group.

18. Sodium benzoate had no teratogenic effect on chicken embryos after injection into the air cell of eggs on day 4 of incubation at levels up to 5mg/egg (JECFA, 1996).

Observations in humans

19. Wide variation in human tolerance has been observed, with gastrointestinal disturbances induced at levels of 5.7g sodium benzoate in some individuals while others tolerated 25-40g. Up to 12 g of benzoic acid

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given over 5 days produced gastric burning and anorexia in 30% of subjects. Acute toxicity seems to be readily reversible and is likely to be due to disturbances in acid-base balance rather than tissue damage.

20. Six men given 0.3-0.4g benzoic acid in their diet for up to 62 days showed no adverse effects and no abnormalities in their blood and urine chemistry or nitrogen balance. Nine patients on penicillin treatment received 1200 mg benzoic acid daily with no adverse effects observed in general or in endogenous creatinine clearance or urine analysis.

21. Oral doses of 250mg/kg bw/day sodium benzoate were given therapeutically to infants to counteract the effects of urea cycle disorders. Doses mixed with food produced no adverse effects, however when given as a bolus dose at the same level, vomiting was induced. Similarly, doses of 125-1000mg/kg bw/day given orally in four divided doses produced anorexia and vomiting in all subjects in the highest dose group and vomiting in those receiving 900 mg/kg bw/day. In one infant, glycosuria, hypocalcaemia and metabolic acidosis were observed after doses of 1000mg/kg bw/day. Accidental treatment with 800mg/kg bw over 24 hours caused acute benzoate poisoning with vomiting, hyperpnoea and irritability. A 19 year old woman experienced generalised itching, flushing, angioedema, dyspnoea and severe hypotension following consumption of cheese containing sodium benzoate. Oral challenge with 20mg sodium benzoate induced mild recurrence of these symptoms

22. Some patients who suffer from asthma, rhinitis or urticaria experience exacerbation of symptoms after consuming benzoates at normal dietary levels. (JECFA, 1996).

JECFA conclusions

23. The committee considered that idiosyncratic reactions reported were not relevant for the general population, or for the establishment of an ADI for this group of compounds. The committee was satisfied that the data available demonstrated a lack of potential for carcinogenic activity or developmental and reproductive toxicity. The ADI of 5 mg/kg bw set at a previous meeting was confirmed. This was derived from a long term carcinogenicity study in rats.

Studies published since the JECFA assessments

Sorbic acid and its salts

Long term studies

24. Groups of Jcl/ICR male mice (5-6/group) were provided with a control diet or diets containing potassium sorbate at doses of 2010, 10050 or 30150 mg/kg bw/day or sorbic acid at 22500 mg/kg bw/day for 12 months. In the high dose potassium sorbate group, body weights were significantly reduced and in the sorbic acid group, the relative body/liver weight ratio was

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significantly increased. Mice dosed with 22500 mg/kg bw/day showed a 40% depletion of hepatic glutathione levels compared to controls after 3 months which persisted until the end of the study. The contents of the small and large intestines were tested for mutagenicity using a reverse mutation assay (as described in table 1. Positive results were obtained for mice dosed with 22500 mg/kg bw/day sorbic acid (~15% of the diet). The Authors concluded that the high incidence of hepatomas described in a previous study (Ishizama et al, 1980) was possibly attributable to glutathione depletion and the production of mutagens in the intestines of the mice. The doses used in this initial study by Ishizama *et al* were very high (15% in the diet). We have been unable to obtain a copy of the Ishizama paper (Tsuchiya & Yamaha, 1984).

25. Groups of Wistar rats, (48 per sex per group) were provided with a diet containing 0, 1.5% and 10% sorbic acid (equivalent to 630 and 4330 mg/kg bw/day in males and 850 and 5690 mg/kg bw/day in females) for 2 years.

26. No differences in bodyweight gain were seen between the control and 1.5% dose groups or food and water consumption between test and control groups. In the 10% dose group the following statistically significant effects were seen compared to controls: 1) reduction in body weight gain from week 26 onwards in females and week 39 onwards in males, 2) a reduction in leukocyte count was observed in females at week 27, but no similar effects were seen in males at the same time or in both sexes at later examination, 3) an elevation in urea concentration was noted in male rats (this was attributed to two elevated results; when these were removed, the mean value returned to a level equivalent to controls), 4) an increase in thyroid weights was observed in male rats, 5) higher relative liver weights were observed in both sexes and higher relative kidney, small intestine and ovary weights were observed in females, 6) an increase in incidence of focal necrosis of the liver was observed in females, but the incidence of bile duct hyperplasia was reduced. No association between dose and tumour formation was observed, with no tumours found in the high dose female group.

27. The authors concluded that no carcinogenic effects were observed in this study. Due to minor changes in the liver in the high dose group, the authors concluded that the NOAEL was 1.5% sorbic acid in the diet giving a dose of approximately 750 mg/kg bw/day (Gaunt et al., 1975).

28. Groups of 48 male and 50 female mice were given sorbic acid in their diets at levels of 0, 1%, 5% and 10% (equivalent to 1500, 7500 and 15000 mg/kg bw/day) for 80 weeks. Control animals were given a mixture of corn oil and starch to ensure that the calorific contents of the test diets were equal. Blood samples were taken throughout and at the end of the study. Hb and WBC counts were measured. Deaths occurred in all groups of mice but did not show signs of being dose-related. Bodyweights were lower in the males of the high dose group through out the study and at autopsy the bodyweights were significantly lower than the control group in males given the 5% and 10% sorbic acid diets and in females given the 10% diet. Liver weights in females in the 5% and 10% sorbic acid groups were significantly increased compared to controls. When expressed relative to body weight, statistically significant

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increases in weights of the brain, liver, kidney, stomach and small intestine were observed in males given 5% and 10% sorbic acid. In females, heart and liver in all dose groups and brain, small intestine, kidney and spleen weights in the highest dose group were increased compared to controls. No dose relationships were found between type and incidence of tumours and tumour incidence was not significantly different to controls. The authors concluded that no evidence of carcinogenicity was found at doses of up to 15000 mg/kg bw/day and that the NOAEL for this study was 1500 mg/kg bw/day because of the minor effects noted at the higher doses (Hendy *et al.*, 1976).

29. Groups of approximately 13 four week old male Jcl-ICR mice were given sorbic acid at an estimated dose of 22500mg/kg bw/day or potassium sorbate at estimated doses of 2010, 10050 and 30150 mg/kg bw/day in the diet for 12 months; a control group was administered a normal diet. The high dose levels were equivalent in terms of sorbic acid concentration. Mice were housed in dose groups and pooled urine was collected. Column chromatography was carried out on the urine samples from each dose group. Lipid peroxides in liver tissue were measured using 2-thiobarbituric acid (TBA) conversion to malondialdehyde. Urinary volume tended to increase and pH decrease in the 22500 mg/kg bw/day sorbic acid group. Following centrifugation and filtration, urine samples were assayed. Levels of lipid peroxidation were consistently lower in the sorbic acid group when compared to controls. In the potassium sorbate groups, there appeared to be a linear reduction relationship between the level of lipid peroxidation and the dose of potassium sorbate (Tsuchiya and Yamaha, 1983).

Benzoic acid and its salts

30. No new relevant studies on benzoic acid and its salts have been identified since the JECFA assessment in 1996.

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Tsuchiya T. and Yamaha T. (1983) Urinary excretion of mutagens and the effects of sorbic acid on the lipid peroxide level in the mice fed on a 15% sorbic acid diet. *Journal of Toxicological Sciences* 8: 213-222

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Annex 3

COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

STUDY ON THE MUTAGENICITY OF SODIUM BENZOATE AND POTASSIUM SORBATE

Method for systematic review

The primary focus for the information present in this paper were the study carried out by Prof. Piper (enclosed as Annex 1) and the two JECFA reviews which can be located using the links provided in the bibliography.

The remainder of the studies were found using Pub-Med as supplied by the US National Library of Medicine and the National Institutes of Health. The search criteria for each search included limits on the dates which excluded studies published prior to 01/01/1994 in the case of benzoic acid and its salts and studies published prior to 01/01/1971 in the case of sorbic acid. This excludes most studies that were available to JECFA at the time of their reviews on these two groups of substances with an overlap to allow for studies that may have been missed in the latter stages of the JECFA assessments.

Search terms used:

Benzoic (Limits: In Vitro, English, Toxicology)

Benzoic Safety (Limits: Humans, Animals, English, Toxicology)

Benzoate (Limits: In Vitro, English, Toxicology)

Benzoate Safety (Limits: Humans, Animals, English, Toxicology)

Sorbate (Limits: In vitro, English, Toxicology)

Sorbate (Limits: Humans, Animals, English, Toxicology)

Sorbic acid (Limits: In vitro, English, Toxicology)

Sorbic acid (Limits: Humans, Animals, English, Toxicology)

Oxidative stress, benzoate

Oxidative stress, benzoic acid

Oxidative stress, sorbate

Oxidative stress, sorbic acid

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Toxicology studies were identified from the search results and those in vivo studies using a non-oral method of administration were excluded from the search.

Additionally, a number of reviews were used to look for relevant references and these were:

Andersen A. (2006) Final report on the safety assessment of benzaldehyde. *Int J Toxicol.* 25 (1) 11-27

Nair B. (2001) Final report on the safety assessment of benzyl alcohol, benzoic acid and sodium benzoate. *Int J Toxicol.* 20 (3) 23-50

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