TOXICOLOGICAL ASPECTS OF SORBATES: A REVIEW

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ABSTRACT

Sorbic acid its sodium or potassium salts (sorbates) are still commonly used in preserved foods of plant or animal origin. It effectively inhibits the growth of yeast and mold species in processed foods pose no health hazard when properly used. According to published literatures, it is clear that sorbates are less toxic than other commonly used antimicrobial agents, because sorbic acid is metabolized in mammals in a similar way as other fatty acid such as caproic acid. For comparison, the LD₅₀ of sodium chloride in rats is 5 g/kg body weight, while the LD₅₀ of sorbic acid and potassium sorbate is 10.5 and 6.2 g/kg body weight, respectively. Therefore, consumers, food processors and regulatory agencies should not consider sorbates as the most dangerous "public enemy". Proper method and levels of use should always be advised.

INTRODUCTION

One of the reasons for the increased use of chemical preservatives has been the changes in the ways foods are produced and marketed. Today’s consumers expect all foods not only to have good taste and nutritious, but also available year-round, to be free of foodborne pathogens, to have a reasonably long shelf life, and affordable. While developments in food processing and packaging systems capable of extending the shelf life without added chemicals, antimicrobial food preservatives still play a significant role in protecting the food supply especially in developing countries where refrigeration system is lacking.

Several factors must be considered when choosing an appropriate antimicrobial agent. These factors include: (a) range of antimicrobial activity, (b) physical and chemical properties, (c) interaction with other processing techniques as well as with other food ingredients, and (d) its safety or toxicity and legal status. In the case of most food additives we are dealing with substances which have a very low toxicity in any living species but with antimicrobial agents the situation is different, because the substances are biocidal to the targeted microorganisms. Therefore, it is necessary to demonstrate that it toxicity to the intended species is highly selective and that no toxic hazard to humans under the approved conditions of use.

One of commonly used antimicrobial agents which is relatively cheap, readily available and applicable for various processed foods especially in developing countries is sorbic acid and its potassium or sodium salts. Sorbic acid is a trans-trans-2,4-hexadienoic acid with a molecular formula C₆H₈O₂ and a molecular weight of 112.14. Usually sorbic acid is colorless or white powder with a characteristic odor and forms a sol in hot water as well as in alcohol and ether. It is frequently used in baked goods, beverages (carbonated or still), bread, cake batters, cake fillings, cake topping, cheese, cottage cheese (creammed), fish (smoked or salted), fruit juices (fresh), fruits (dried), margarine, oleomargarine, pickled goods, pie crusts, pie fillings, salad dressings, salads (fresh), sausages (dry), sea food cocktails, syrups (chocolate dairy), and wines (Lewis, 1989).

Sorbic acid and its potassium, sodium or calcium salts are collectively known as sorbates. Sorbates are permitted antimicrobial additives in many countries including United States of America, United Kingdom and other European countries (Table 1). The antimicrobial activity of sorbic acid is greatest when the compound is in the undissociated state. The pKₐ of sorbic acid is 4.75; therefore, antimicrobial activity is greatest as the pH decreases and is essentially non-existent at pH higher than 6.5 (Walker, 1990). Food-related yeasts inhibited by sorbates include species of Brettanomyces, Byssochlamys, Candida, Cryptococcus, Debaryomyces, Endomycopsis, Hansenula, Oospora, Pichia, Rhodotorula, Saccharomyces, Sporobolomyces, Torulaspora, Candida, and Zygosaccharomyces (Sofos, 1989). Food-related mold species inhibited by sorbates belong to the genera Alternaria, Ascochyto, Aspergillus, Botrytis, Chepalosporium, Fusarium, Geotrichum, Gliocladium, Helminthosporium, Humicola, Mucor, Penicillium, Phoma, Pullularia (Auebasidium), Sporotrichum, and Trichoderma (Sofos, 1989).

The objective of this paper is to review the toxicological aspects of sorbic acid and sorbates as antimicrobial agent.
<table>
<thead>
<tr>
<th>Types of Food</th>
<th>Canada</th>
<th>France</th>
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<th>Japan</th>
<th>U.K.</th>
<th>U.S.A.</th>
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<td>200 – 300 ppm</td>
<td>200 ppm</td>
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<td>Tea, coffee, chocolate</td>
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- : No regulations exist in that country regarding particular use of this additive.
* : Authorized without limitation.
** : Good manufacturing practices.
ACUTE TOXICITY

The oral LD₉₀ of sorbic acid in rats is in the range of 7.4 to 10.5 g/kg body weight. For potassium sorbate, the oral LD₉₀ in rats is 4.2 to 6.2 g/kg body weight, and for sodium sorbate 5.9 to 7.2 g/kg body weight (Deuel et al., 1954a; Smyth and Carpenter, 1948). For comparison, the acute toxicity (LD₉₀) of sodium chloride is 5 g/kg body weight (Luck, 1976). Considering these high acute toxicity doses, sorbates are believed to be less toxic than other common food preservatives.

SUBCHRONIC TOXICITY

Administration by oral intubation of 40 mg sorbic acid per kilogram body weight per day to groups of male and female mice for 2 months resulted in similar survival, food composition, and weight-gain rates between treated and control animals (Shitenberg and Ignat'ev, 1970). When feed intake was restricted by 50% at the end of the study for 5 days, the mortality rate and weight loss of the treated mice were less than those of the controls. However, administration of 80 mg sorbic acid/kg body weight/day for 3 months resulted in somewhat restricted growth compared to control mice. Restriction of feed intake by 90% for 18 days caused no difference in mortality rates between treated and control animals (Shitenberg and Ignat'ev, 1970).

Feeding two strains of rats with diets containing 4% or 8% sorbic acid for 90 days also had no effect on the rate of weight gain (Deuel et al., 1954a). However, another study by Demaree et al. (1955) found that incorporation of 10% sorbic acid in the diet did not alter the quantity of feed consumed by rats, but some of the test animals showed a higher growth rate. Groups of rats were fed varying levels (up to 10%) of potassium sorbate for a period of 3 months. The level of 10% sorbate depressed body weight gain initially, while the 5% level had a similar but lesser effect on the female rats. At the end of the study, the weights of the rats fed with diet containing 10% sorbate were slightly depressed, but their feed consumption had also been smaller. Thus, the feed efficiency (i.e., weight gain per gram of feed) was similar in both groups (Demaree et al., 1955).

Feeding dogs with diets containing 1% or 2% potassium sorbate for 3 months caused no adverse effect on the animals. Dogs fed for 3 months a diet containing 50% cheddar cheese with 4% sorbic or caproic acids showed no adverse effects compared to dogs fed the same cheese diet without sorbate (Deuel et al., 1954a).

Sorbic acid (3.3 g daily/kg body weight) was also tolerated well by rabbits (Kuhn et al., 1937).

One effect observed in some feeding studies has been an enlargement of the liver with use of sorbic-acid-containing diets (Demaree et al., 1955; Gaunt et al., 1975; Hendy et al., 1976). The histopathological appearance of the liver, however, have been normal. This increase in liver weight has been interpreted as functional hypertrophy and has been attributed to the caloric utilization of sorbic acid (Smyth and Carpenter, 1948; Lang, 1960). Increased kidney weights in animals fed diets containing potassium sorbate (5 to 10%) have been attributed to the high potassium levels. Gross pathological examination, however, has shown no abnormalities.

CHRONIC TOXICITY

Mice or rats fed with diets containing 10% sorbic acid for 2 years resulted in somewhat increased thyroid weights in the males; lower body weight in both sexes; and increased kidney, small intestine, and ovary weights in the females (Gaunt et al., 1975; Hendy et al., 1976). The sorbic acid content in the diet of rats corresponds to an approximately daily intake of 150 to 200 g sorbic acid by human adults, which is unrealistically extreme (Luck, 1976).

Rats fed with diets containing 5% sorbic acid during their life-span showed no signs of any damage, and all their bodily functions examined remained normal (Lang, 1960). The male rats, however, had a tendency for accelerated growth and showed an increased life-span. These effects have been attributed to the higher caloric supply due to sorbic acid in the diet and to a lowering of the premature mortality rate, caused by sorbic acid through improved resistance to infections (Luck, 1976; Luck, 1980).

Rats and mice fed with 40 to 90 mg sorbic acid/kg body weight for a period of up to 18 months did not show any detrimental effects (Shitenberg and Ignat'ev, 1970). Other study using rats fed with 0.1% potassium sorbate in their diets and 0.3% potassium sorbate in their drinking water for 60 weeks showed satisfactory health and survival rates (Dickens et al., 1966). No abnormalities were observed in liver, kidney, hearth, and testes of rats fed with 5% sorbic for their life-span (Lang, 1960).
REPRODUCTIVE TOXICITY

No adverse effects on reproductive function of post-natal development were observed in a four-generation study in mice receiving sorbic acid and nisin at dose levels of 40 mg/kg body weight/day and 2 mg/kg body weight/day, respectively. The mice were given the test diet for 8 months and were observed for 3.5 months post-weaning. In the F1 generation the weight gain of the treated offspring was better than controls, although this is of doubtful significance (Sh滕berg and Ignat’ev, 1970).

Similarly, in rats, sorbic acid at a dietary level of 10% was without effect on reproductive performance when the parenteral generation was exposed for 60 days prior to mating and throughout gestation and lactation. The F1 generation were weaned on to the test diet for 70 days then mated. No significant difference was observed between test and control animals in reproductive parameters. However, males in the treated group showed a small reduction in weight gain (Demaree et al., 1955).

OBSERVATION IN HUMANS

Sorbic acid is known to be associated with a non-immunological contact urticaria (Hannukela and Haahstela, 1987) and a population subgroup who are especially sensitive to lactic acid (referred to as 'stingers' because of the stinging sensation elicited by the acid) have also been found to be unusually sensitive to sorbic acid in objective and immediate non-immunological contact urticaria test. In this test the 'stingers' developed significantly more erythema in response to 0.5% sorbic acid, and more edema in response to 1% sorbic acid (Lammintausta et al., 1988). In clinical study of 90 patients with chronic or chronic relapsing urticaria, 4% were responsive to sorbic acid and/or other food additives.

There are other reports of sorbic acid being associated with pseudo-allergy and with the so-called 'burning mouth syndrome' (Lamey et al., 1987; Haustein 1988). The overall incidence appears to be low but further work is needed to clarify this (Walker, 1990).

METABOLISM

The lack of toxicity of sorbic acid can be explained by the fact that it is metabolized in mammals in a similar manner to other fatty acids such as caproic acid, and can thus serve as an energy source. In the presence of adequate metabolizable carbohydrate the major end-products are carbon dioxide and water. If animals are deprived of carbohydrate then high doses of sorbic acid can induce ketosis, the products of metabolism including acetooacetate and acetone, as with caproic acid (Walker, 1990). Sorbic acid at dietary levels up to 5% did not act as antimitabolite for essential fatty acids (Deuel et al., 1954a,b).

Being similar to common dietary fatty acids, the metabolic breakdown of sorbate includes activation by linkage to coenzyme A; hydration by crotonase to a β-hydroxy acids; hydration to a β-keto acid; and cleavage by β-keto-thiolase (Deuel et al., 1954b). The first reaction step (i.e., α, β-dehydrogenation) of β-oxidation is omitted because sorbic acid already has an α,β-double bond (Kuhn et al., 1937; Deuel et al., 1954b). Thus, sorbic acid is an intermediate in the metabolism of caproic acid and follows its pathway of degradation in the animal and human body. In cases of very high levels of sorbate, there is evidence of ω-oxidation of sorbic acid, similar to that of other fatty acids (Deuel et al., 1954b).

Thus, sorbic acid can be used by animal organisms as a source of calories, because it is metabolized in a manner similar to its saturated counterpart, caproic acid (Deuel et al., 1954a,b). In vitro investigation have also indicated that sorbic acid is metabolized like a fatty acid and yields 6.6 kcal/g, 50% of which is biologically utilisable (Luck, 1980).

The process of decomposition has been followed in vitro with sorbic acid labeled with radioactive carbon-14 (14C). The results have shown that the compound is almost entirely absorbed from the intestine and about 85% of the total amount is oxidized in a period of a few hours. The half-life depends on initial dosage and is in the range of 40 to 110 min (Fingerhut et al., 1962). Although 85% of the initial sorbic acid was metabolized and given off as carbon dioxide, 3% was found in internal organs, 3% in skeletal muscle, 2% in the urine as urea and carbon dioxide, 0.4% in the feces, and 6.6% in other parts of the body (Fingerhut et al., 1962). Radioactivity found in lipid fractions showed that part of the acetyl coenzyme A formed from the degradation of sorbate was used for synthesis of new fatty acids. Studies with mice also showed that 81% of the radioactivity was released as carbon dioxide, and about 4% was found in the urine as muconic and sorbic acids (Westoo, 1964). In general, sorbate is readily metabolized in animals and human under the metabolic mechanisms for fatty acids.
On the basis of the finding that sorbic acid (SA)-induced hepatoma was correlated with the depletion of reduced glutathione (GSH) in mouse liver (Tsuchiya and Yamada, 1984), the possible conversion of sorbic acid to a metabolite which is reactive with SH-compounds was investigated by Nishimaki-Mogami et al., (1991). They indicated that sorbyol-CoA was hydrated and then reduced to 3-keto-4-hexenoyl-CoA by the combined actions of mitochondrial hydratase (crotonase) and L-3-hydroxyacyl-CoA dehydrogenase. Upon the addition of GSH or coenzyme A, 3-keto-4-hexenoyl-CoA was nonenzymatically converted to another 3-ketoacyl-CoA derivative in a time- and concentration-dependent manner.

Two-weeks feeding of mice of 15% sorbic acid caused a 2.0-fold induction of peroxisome β-oxidation in the liver (Nishimaki-Mogami et al., 1991). Sorbic acid caused a marked induction (3.6 fold) of hydratase toward sorbyol-CoA but a less pronounced induction (1.3-fold) of 2,4-dienoyl-CoA reductase, leading to about a 3-fold elevation in the hydratase: reductase ration. The elevated ratio was sustained throughout the period of sorbic acid feeding up to 12 weeks. Therefore, a large amount of sorbic acid could be converted to 3-keto-4-hexenoyl-CoA during this period (Nishimaki-Mogami et al., 1991). Oxidative stress caused by a depleted cellular SH-pool together with the induction of peroxisome proliferation by sorbic acid-feeding may implicate the mechanism by which non-mutagenic sorbic acid caused hepatoma (Nishimaki-Mogami et al., 1991).

GENOTOXICITY

A paper by Hasegawa et al. (1984) raised concern about a potential genotoxic hazard of sorbic acid and its salts. It was reported that sodium sorbate induced in vitro chromosome aberrations, sister chromatid exchanges, and gene mutations in cultured Chinese hamster V79 cells. These studies supported earlier findings by Ishidate and Odashima (1977) and Abe and Sasaki (1977) which also indicated a clastogenic effect of potassium sorbate in vitro but were not widely appreciated. Sodium sorbate (0.4 – 0.8 mg/mL) was found to induce sister chromatid exchanges, chromosome aberrations and 6 thioquanine-resistant mutations in Chinese hamster V79 cells in vitro (Hasegawa et al., 1984). The clastogenic potency of sodium sorbate was estimated to be 180 times lower than that of the positive control methyl-N'-nitro-N-nitrosoguanidine. Sorbic acid and its potassium salt, however, turned out to be much less clastogenic/mutagenic and showed significant effects only at the highest concentrations tested (1 and 20 mg/mL, respectively). It was thought that these effects were due to the high concentrations which increased the osmotic pressure of the culture medium and subsequently influenced cell metabolism (Galloway et al., 1987).

While the majority of investigation have not revealed mutagenic properties of sorbic acid and its salts, some recent reports have indicated a possible genotoxic potential of these compounds. Namiki and Kada (1975) observed that sorbic acid was effective in the rec-assay at a concentration of 2-4 mg/mL. From the results of Abe and Sasaki (1977) and Ishidate and Odashima (1977) it is evident that potassium sorbate has been implicated having clastogenic effects, sister chromatid exchanges, and translocations in Chinese hamster V79 cells.

Sorbic acid and potassium sorbate were less genotoxic that the sodium salt. Furthermore, sodium sorbate showed weak mutagenicity in the Ames tester strain TA100 without S-9 mix. Tsuchiya and Yamada (1983a,b) found Ames-positive substances in feces and urine of mice fed a diet containing 15% sorbic acid or potassium sorbate. More recently Mukherjee et al. (1988) reported that sorbic acid induced sister chromatid exchanges and micronuclei formation in mice after intraperitoneal injection at high doses (150 mg/kg body weight).

Munzner et al. (1990) investigated the possible genotoxic actions of potassium sorbate and sodium sorbate using the Salmonella/mammalian-microsome test, hypoxanthine-guanine-phosphoribosyl transferase and sister chromatid exchange test with Chinese hamster ovary cells, the micronucleus test on bone marrow cells of mice and Chinese hamsters, and the chromosome aberration and sister chromatid exchange test of Chinese hamsters. In all of the in vitro tests no signs of genotoxicity were detected. No in vivo mutagenicity effect was observed when freshly prepared potassium sorbate, sodium sorbate or stored potassium sorbate (200 mg/kg body weight) were used. Investigations with stored sodium sorbate revealed weak clastogenic activity by increased chromosome aberrations and elevated numbers of micronuclei at doses of 200 mg/kg body weight, but no induction of sister chromatid exchange was found (Munzner et al., 1990).

Fresh sodium sorbate solution at 2.5 mg/mL (18.6 mM) had a toxic effect and arrested V79 cells at the G1/M phase (Schlatter et al., 1992). This effect can be attributed to the extreme culture conditions (Scott et al. 1991; Schlatter et al., 1992). Solutions of both the
sodium and the potassium salts, stored for 28 days at room temperature, had a strong cytotoxic effect, arrested the cycle and increased cellular protein content in V79 cells (Schlatter et al., 1992). Stored potassium sorbate increased the coefficient of variation of the DNA distribution of G1 cells, indicating clearly a disturbance of the chromosome segregation in mitosis. Comparing potassium sorbate stored for 4 weeks at room temperature with freshly prepared solutions in Chinese hamster ovary cells Munzer et al. (1990) found a decreased survival at concentrations 10 – 20 mg/mL.

Schiffmann and Schlatter (1992) tested the genotoxic potential of sorbic acid, sodium sorbate, and potassium sorbate in Syrian hamster embryo fibroblast micronucleus assay and the Syrian hamster embryo cell transformation test in vitro. Sorbic acid and potassium sorbate up to 1200 µg/mL showed no activity in either test system. When freshly prepared sodium sorbate solutions were used, no genotoxic or cell-transforming activity was detected. However, sodium sorbate which previously has been heated, sonicated to facilitate solubilization, and stored for 48 hr at room temperature yielded a positive response in both test systems. It is concluded that oxidation products of sodium sorbate that posses genotoxic and cell-transforming properties are formed under conditions of heating, sonication, and storage (Schiffmann and Schlatter, 1992).

Jung et al. (1992) also studied the genotoxic potential of very high doses of sorbic acid and potassium sorbate using the sister chromatid exchange and micronucleus tests. In addition, the potential of these compounds to induce DNA damage/repair was investigated using an in vitro unscheduled DNA synthesis test and an in vitro and in vivo alkaline elution techniques. Oral administration of sorbic acid (up to 5000 mg/kg body weight) did not induce sister chromatid exchanges or the formation of micronuclei in bone marrow cells of mice. Intraperitoneal treatment of rats with 400 – 1200 mg potassium sorbate/kg body weight did not alter the elution profile of DNA from isolated liver cells in the in vivo alkaline elution assay. Sorbic acid did not induce DNA repair in cultured human A549 cells in the unscheduled DNA synthesis test. In vitro incubation of the cells with 1 – 1000 µg potassium sorbate/mL in the absence or presence of rat liver homogenate did not result in the formation of DNA single-trand breaks in the alkaline elution assay. These results demonstrate that sorbic acid and its potassium salt are not genotoxic in vivo or in vitro. In contrast to sorbic acid and potassium sorbate, sodium sorbate is very sensitive to oxidative degradation and its main oxidation product was identified to be 4,5-oxohexanoate, which was mutagenic in the Ames test (Jung et al., 1992). Although the genotoxicity status is still unclear, Würger et al. (1992) suggested that sodium sorbate should be deleted from the list of permitted food preservatives.

**SUMMARY**

Sorbic acid and sorbates are still commonly used in wide variety of processed foods not only in developing countries, but also in developed countries. It is one of antimicrobial agents which effectively inhibits the growth of yeast and mold species. Consumers, food processors and regulatory agencies should not considered sorbates as one of the "public enemies". According to published information discussed in this paper, it is clear that sorbates are less toxic than other commonly used antimicrobial agents, because sorbic acid is metabolized in mammals in a similar way as other fatty acid such as capric acid. For comparison, the LD50 of sodium chloride in rats is 5 g/kg body weight, while the LD50 of sorbic acid and potassium sorbate is 10.5 and 6.2 g/kg body weight, respectively. That means, sorbates is less toxic than sodium chloride, but users should always be informed about the safe level of use in foods.

**REFERENCES**


