参考資料２－１

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EVALUATION OF CERTAIN FOOD ADDITIVES

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or humans. Taking into account data showing that lutein was not genotoxic, had no structural alert, did not exhibit tumour promoting activity, and is a natural component of the body (the eye), the Committee concluded that there was no need for a study of carcinogenicity.

Lutein has some structural similarities to β-carotene, which has been reported to enhance the development of lung cancer when given in supplement form to heavy smokers. The available data indicated that lutein in food would not be expected to have this effect. The Committee was unable to assess whether lutein in the form of supplements would have the reported effect in heavy smokers.

The 52-week study in monkeys was designed to evaluate ocular effects, and although there were no adverse toxicological effects at the highest dose tested (20 mg/kg bw per day), this study was considered to be inappropriate for the establishment of an ADI, in view of the much higher doses used in several other studies and found to be that without effect. The available comparative toxicokinetic data for humans and rats indicated that the studies of toxicity in rats could be used to derive an ADI. The Committee concluded that the best study for this purpose was the 90-day study in rats. An ADI of 0–2 mg/kg bw was allocated based on the NOEL of 200 mg/kg bw per day (the highest dose tested in this study) and a safety factor of 100.

Although the ADI was based on the results of a short-term study, the supporting data and lack of effects at much higher doses in some studies (e.g. a study of developmental toxicity), indicated that the safety factor of 100 was appropriate.

In view of the toxicological data and structural and physiological similarities between the xanthophylls lutein and zeaxanthin, the Committee decided to include zeaxanthin in the ADI (0–2 mg/kg bw) for lutein, which had a stronger toxicological database, and to make this a group ADI for these two substances. This group ADI does not apply to other xanthophyll-containing extracts with a lutein or zeaxanthin content lower than that cited in the specifications.

A toxicological monograph, a Chemical and Technical Assessment, and specifications were prepared for lutein from *Tagetes erecta*.

3.1.5 *Peroxyacid antimicrobial solutions containing 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP)*

Explanation

The Committee considered the safety of antimicrobial solutions that are prepared from acetic acid and octanoic acid (singly or in

combination), together with hydrogen peroxide, and using 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP) as a sequestrant or stabilizer. Preparations that are ready for use also contain as active compounds the peroxy forms of both acids. Before use, concentrated solutions are diluted to achieve target concentrations of total peroxyacid ranging from 80 to 200 mg/kg. These antimicrobial solutions are intended for use as components of wash solutions on fresh poultry and meat and in wash water for fresh and processed fruits and vegetables. After being applied in process water, they are largely eliminated by drainage, further washing and trimming of products, and evaporation. The safety of the antimicrobial solutions was therefore assessed on a component-by-component basis, considering the potential residue of each component or its breakdown products in food as consumed.

At its seventeenth meeting (Annex 1, reference *32*), the Committee allocated an ADI "not limited"[1] to acetic acid and its potassium and sodium salts. This ADI was retained at the forty-ninth meeting (Annex 1, reference *131*) when the Committee evaluated a group of flavouring agents (saturated aliphatic acyclic linear primary alcohols, aldehydes, and acids) that included acetic acid.

At its forty-ninth meeting, the Committee evaluated octanoic acid for use as a flavouring agent as part of the group of saturated aliphatic acyclic linear primary alcohols, aldehydes, and acids, and concluded that octanoic acid posed no safety concerns at intakes of up to 3800 µg/person per day (or 63 µg/kg bw per day, assuming a body weight of 60 kg).

At its twenty-fourth meeting (Annex 1, reference *53*), the Committee evaluated hydrogen peroxide as a preservative and sterilizing agent for use in milk. While an ADI was not allocated, the Committee noted that hydrogen peroxide should be used only when better methods of milk preservation were not available.

Peroxyacetic acid and peroxyoctanoic acid, and HEDP have not been previously evaluated by the Committee.

At its present meeting, the Committee considered a number of studies on the antimicrobial efficacy of peroxyacid solutions, the toxicity of HEDP, and the effects of peroxyacid solutions on food quality and nutritional value. The Committee also evaluated estimates of the intake of the individual components in these solutions for consideration in the safety evaluation.

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[1] A term no longer used by the Committee, which has the same meaning as ADI "not specified".

Chemical and technical considerations

Antimicrobial washing solutions are manufactured by mixing hydrogen peroxide (4–12%), acetic acid (40–50%), and HEDP (<1%), with or without octanoic acid (3–10%). The concentrations of peroxyoctanoic acid and peroxyacetic acid at equilibrium are in the range of 1–4% and 12–15%, respectively. Peroxyacetic acid was identified as the main antimicrobial substance in the washing solutions. Information submitted indicated that reductions of naturally-occurring microbial flora on treated food were generally less than one order of magnitude. Peroxy compounds in washing solutions used on food were reported to break down rapidly to water, oxygen, acetic acid and octanoic acid. Estimated residues of HEDP and octanoic acid in treated food were ≤0.2 mg/kg and ≤4 mg/kg, respectively. Because the peroxy compounds are highly reactive, these compounds will not leave residues on food and consumers will not be exposed to these substances.

It was noted that there were limited studies available on the effects of the washing solutions on nutrients and there were no studies that identified residues of reaction products that might be formed by the reaction of peroxy compounds with the food components.

The existing specifications for acetic acid, glacial, prepared by the Committee at its nineteenth meeting (Annex 1, reference *38*) were revised to include details of the materials and methods of its manufacture. The maximum limit for lead was reduced to 0.5 mg/kg to be consistent with other monographs for acetic acid, considering the broad use in the food industry. The existing specifications for hydrogen peroxide were initially elaborated in conjunction with the evaluation of the lacto-peroxidase milk sterilization system, carried out by the Committee at its twenty-ninth meeting (Annex 1, reference *70*). They were revised to include details of the materials and methods of manufacture. The functional use descriptor was revised to "antimicrobial agent" to reflect function. The Committee decided to prepare new specifications for octanoic acid as a food additive because of a lower minimum assay value requirement than that listed in the specifications for this compound as a flavouring agent. Specifications for octanoic acid as a flavouring agent were prepared by the Committee at its forty-ninth meeting (Annex 1, reference *131*). New specifications for HEDP, as sequestrant and stabilizer for peroxy-based antimicrobial washing solutions, were also prepared.

Intake

The Committee evaluated estimates of intake of each component used in the peroxyacid solutions on the basis of residual amounts

anticipated to be present on treated food at the time of consumption. Consistent with what was known about the chemistry of peroxy compounds, no residues of hydrogen peroxide, peroxyacetic acid, or peroxyoctanoic acid were anticipated to be present on foods that have been washed in, sprayed with, or otherwise treated using these solutions.

Acetic and octanoic acid present in the solutions and as by-products from the corresponding peroxyacids would be expected to remain on any treated foods that are not washed or further processed after treatment. The Committee noted that the estimate of exposure to octanoic acid resulting from the use of the antimicrobial solutions, 1.9 mg/day, was highly conservative. The mean intake of octanoic acid from foods consumed as part of the diet in the USA was estimated to be approximately 200 mg/day. Intake of acetic acid was not explicitly analysed, but its use in and on foods (vinegar) would result in a greater exposure than that from the use of peroxyacid antimicrobial solutions. The Committee did not further consider exposure to these common food acids.

HEDP is expected to remain on foods that are treated with antimicrobial solutions and that are not further washed, processed, or cooked. The highest estimate of intake of HEDP prepared using GEMS/Food diets was that for the European diet: 3.6 µg/kg bw per day for the upper-bound estimate using a model for vegetables with a high surface area. The Committee also considered national estimates of intake from the Czech Republic, the USA, and the United Kingdom. The upper-bound estimate of exposure was 2.2 µg/kg bw per day for the Czech Republic. The mean and 90th percentile upper-bound estimates of intake for the USA were 2.2 and 4.7 µg/kg bw per day, respectively. The mean and 90th percentile upper-bound estimates of intake for the United Kingdom were 1.8 µg/kg bw per day and 3.3 µg/kg bw per day, respectively.

The Committee was aware of the non-food uses of HEDP. It is used as an anti-scalant for water treatment and in boilers worldwide (the regulatory limit for this use is 25 µg/l in the USA). HEDP is also used as a drug to treat Paget disease, and in some over-the-counter cosmetic and pharmaceutical formulations. The United States Environmental Protection Agency (EPA) estimated that exposure to HEDP from all these uses was not more than 6 µg/kg bw per day, including 0.04 µg/kg bw per day from its use on food (*3*). The Committee noted that this estimate of exposure resulting from food uses of HEDP was much less conservative than that used in the present evaluation.

Antimicrobial efficacy

Information available to the Committee indicated that peroxyacetic acid solutions enhance the action of water sprayed on food surfaces to reduce numbers of bacteria. While reductions in numbers of microbes were demonstrated, some of the data provided suggest that the results of replicate tests were rather inconsistent, with standard deviations close to or greater than the value of the reductions themselves. Testing of food surfaces showed modest reductions in numbers of microbes, when either endogenous microorganisms (represented by total aerobic plate counts) or inoculated ("spiked") pathogens (commonly *Listeria monocytogenes*, *Escherichia coli* O157:H7, and some *Salmonella* serotypes) were monitored. Data from laboratory and in-plant tests indicated that the use of these solutions would minimize the possibility of cross-contamination, although they are unable to remove all adherent viable bacteria from food surfaces.

The Committee did not further consider the antimicrobial efficacy of peroxyacid antimicrobial solutions containing HEDP.

Toxicological data

Antimicrobial solutions are equilibrium solutions that are diluted in water before use in food processing. Hydrogen peroxide in these solutions will dissociate into water and oxygen. Both peroxyacetic acid and peroxyoctanoic acid are also inherently unstable and will breakdown into acetic acid and octanoic acid, respectively, although their stability is enhanced by HEDP. Low residual amounts of these simple organic acids present on food at the time of consumption would pose no safety concern. It is not expected that residues of peroxyacetic acid or peroxyoctanoic acid from these solutions will be present on treated foods at the time of consumption. The peroxide components of the peroxyacid antimicrobial solutions thus pose no toxicological concerns with regard to the uses considered by the Committee. The Committee concluded that HEDP, which sequesters metal ions thereby stabilizing the peroxy compounds in peroxyacid antimicrobial solutions, is the only component of potential toxicological concern.

Data reviewed by the Committee indicated that absorption of HEDP from the gastrointestinal tract is very limited and that its metabolism is negligible. The limited amount of data available to the Committee suggested that absorption may be related to age and species. The skeleton is the target site for the disposition of HEDP in all species.

HEDP did not show evidence of mutagenic activity in assays in five strains of *Salmonella* or in an assay for mutation in L51718 $Tk^{+/-}$

mouse lymphoma cells, with and without metabolic activation from mammalian microsomes.

In two 90-day studies of toxicity, rats were fed diets containing HEDP at doses ranging from 100 to 2500 mg/kg bw per day. The highest dose tested in each study (i.e. 1500 or 2500 mg/kg bw per day) caused mortality and signs of toxicity, but no effects were reported at lower doses in either study. The NOEL was 500 mg/kg bw per day in both studies.

In a 90-day study of toxicity in dogs, HEDP was administered orally at a dose equivalent to 0, 25, 75, or 250 mg/kg bw per day. No adverse effects attributable to treatment were reported. The NOEL for HEDP was 250 mg/kg bw per day. The Committee also evaluated the results of a long-term study to determine the skeletal effects of daily subcutaneous injections of HEDP administered to adult female dogs for varying periods ranging from 1 to 2 years. Some effects on bone parameters were observed at all doses. Profound skeletal effects were associated with the administration of daily subcutaneous doses of HEDP of 2–10 mg/kg bw for 1 year. Spontaneous bone fractures were slightly increased in dogs given daily subcutaneous doses of 0.5 mg/kg bw for 2 years, but no permanent skeletal changes were observed at this dose and healing was normal. No fractures were observed at a daily subcutaneous dose of 0.1 mg/kg bw after 2 years. Assuming that 10–20% of the administered dose were absorbed from the gut in dogs, a subcutaneous dose of 0.1 mg/kg bw per day would correspond to an oral dose of 0.5–1 mg/kg per day. In considering these studies, the Committee noted that 90 days may not be long enough to observe skeletal effects in dogs and that there may be differences in the disposition of HEDP in bone that are related to the route of administration.

In a combined two-generation study of reproductive toxicity and teratogenicity, rats were given HEDP (disodium salt) in the diet at concentrations equivalent to 0, 50 or 250 mg/kg bw per day either during their lifetime or only on days 6–15 of gestation, for two generations. No fetal abnormalities indicative of a teratogenic effect were reported at either dose tested. HEDP was embryotoxic when administered at a dose of 250 mg/kg bw per day during organogenesis. The NOEL for HEDP was 50 mg/kg bw per day.

The effects of HEDP (disodium salt) were determined in a combined study of reproductive toxicity and teratogenicity in rabbits. Two experiments were performed because of the observation of toxicity at the lowest and highest doses, administered by gavage, in the first experiment. In the second experiment, rabbits received HEDP at a

dose of 0, 25, 50, or 100 mg/kg bw per day in the diet, or 100 mg/kg bw per day by gavage. Fetuses from dams receiving HEDP at a dose of 100 mg/kg bw per day by gavage were significantly smaller than those from untreated controls. No fetal abnormalities indicative of a teratogenic effect in rabbits were observed in either experiment. The NOEL was 50 mg/kg bw per day.

Use of HEDP to treat Paget disease

The disodium salt of HEDP, known clinically as sodium etidronate, is administered orally at a starting dose of 5 mg/kg bw per day, for not longer than 6 months, to treat patients with Paget disease. Paget disease is an idiopathic disease characterized by accelerated bone metabolism; fractures and other abnormalities of the bone are common in patients with Paget disease. Owing to its high affinity for solid-phase calcium phosphate, HEDP prevents the growth and dissolution of hydroxyapatite crystals on crystal surfaces of bone. The mechanism of action, however, is not fully understood.

Assessment of the effects on food quality and nutritional value

Limited data on the quality and nutritional value of foods treated with peroxyacid antimicrobial solutions were provided to the Committee. Studies were conducted to determine whether treatment of foods with peroxyacid antimicrobial solutions resulted in significant differences in concentrations of thiobarbituric acid (a measure of rancidity), or in fatty-acid profile testing of raw or cooked poultry products and fresh beef samples, when compared with treatment with water only. No differences were found.

The Committee was aware that studies in the literature indicated potential reactions of hydrogen peroxide with components of food. The Committee noted that such studies are typically conducted using high concentrations and long periods of exposure and that, under the conditions of their intended use, the potential reactivity of peroxyacid antimicrobial solutions is expected to be limited. Studies available to the Committee confirmed the low potential reactivity of two peroxyacid antimicrobial solutions in dilute ready-to-use solutions that are in brief contact with fruits and vegetables.

A study was conducted to determine the effects of peroxyacetic acid and hydrogen peroxide on the content of β-carotene and vitamin C in tomatoes, potatoes and broccoli. These foods were prepared for consumption using "worst-case" exposure conditions, i.e. peroxyacetic acid at 80 mg/kg and hydrogen peroxide at 59 mg/kg for 5 min, and then rinsed. When treated samples were compared with controls, there were no effects on the β-carotene content of tomatoes or

broccoli, on the vitamin C content of potatoes or broccoli, or on the active vitamin C content of tomatoes.

On the basis of the available data, the Committee concluded that peroxyacid antimicrobial solutions are unlikely to have an adverse effect on food quality or nutritional value, with regard to the uses considered by the Committee.

Evaluation

The Committee considered the safety, on a component-by-component basis, of antimicrobial solutions containing HEDP and three or more of the following components: peroxacetic acid, acetic acid, hydrogen peroxide, octanoic acid and peroxyoctanoic acid. These solutions are intended to be diluted before use to achieve peroxyacid concentrations in the range of 80 to 220 mg/kg. The Committee concluded that the peroxy compounds in these solutions (hydrogen peroxide, peroxyacetic acid and peroxyoctanoic acid) would break down into acetic acid and octanoic acid, and that small residual quantities of these acids on foods at the time of consumption would not pose a safety concern. Therefore, the Committee focused its evaluation on the residues of HEDP that are expected to remain on foods treated, in accordance with manufacturers instructions, with peroxyacid antimicrobial solutions that contain HEDP at up to 1%.

The Committee compared the highest estimate of intake of HEDP from the uses of peroxyacid antimicrobial solutions considered by the Committee (i.e. 0.004 mg/kg bw per day) with the starting oral dose used to treat Paget disease (i.e. 5 mg/kg bw per day) and noted that the margin of exposure is >1000. Based on this margin of exposure, the conservative nature of the estimates of intake of HEDP, and the available toxicity data, the Committee concluded that HEDP does not pose a safety concern at the concentrations of residue that are expected to remain on foods.

The Committee noted that the use of peroxyacid antimicrobial solutions does not replace the need for good hygienic practices in handling and processing of food.

A toxicological monograph and new specifications for HEDP and octanoic acid were prepared. Existing specifications for acetic acid and hydrogen peroxide were revised. Chemical and Technical Assessments were prepared for the peroxy-based antimicrobial washing solutions and HEDP.