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WHO FOOD ADDITIVES SERIES: 54

Safety evaluation of certain food additives

Prepared by the
Sixty-third meeting of the Joint FAO/WHO
Expert Committee on Food Additives (JECFA)

IPCS

International Programme on Chemical Safety
World Health Organization, Geneva

PEROXYACID ANTIMICROBIAL SOLUTIONS CONTAINING 1-HYDROXYETHYLIDENE-1,1-DIPHOSPHONIC ACID (HEDP)

First draft prepared by

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1. 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 acceptable daily intake (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

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

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.

1.1 Composition of antimicrobial solutions

The composition of four antimicrobial solutions, A–D, are described in Table 1. The concentrated solutions are diluted before use to achieve target concentrations of total peroxyacid ranging from 80 to 200 mg/kg.

In manufacturing each antimicrobial solution, measured quantities of each component are added in a specific order. Hydrogen peroxide reacts with acetic acid to form peroxyacetic acid, which it thus helps to stabilize. Hydrogen peroxide also reacts with octanoic acid, when present, to form peroxyoctanoic acid. The result is an equilibrium solution containing peroxyacetic acid, acetic acid, hydrogen peroxide, HEDP, and in some cases, octanoic acid and peroxyoctanoic acid. The concentration of peroxyacids continues to increase for 7–13 days after manufacture. HEDP is needed to ensure the stability of the solution since peroxy compounds are inherently unstable. Once equilibrium is achieved, the solution remains relatively stable at room temperature for up to 1 year. The main chemical reactions that occur in the equilibrium solutions are shown in Figure 1.

1.2 Residues of components of antimicrobial solutions

After application, the antimicrobial solutions and their components are largely lost due to drainage, further washing, trimming and evaporation. Residues of hydrogen peroxide, peroxyacetic acid, or peroxyoctanoic acid on food rapidly decompose into water, oxygen, acetic acid and octanoic acid (Figure 1). Small amounts of acetic acid, octanoic acid and HEDP will remain on the treated commodities. Intake assessments for the components of the antimicrobial solutions are described in section 3.

2. BIOLOGICAL DATA

Antimicrobial mixtures are equilibrium mixtures that are diluted in water prior to their use in processing food. Hydrogen peroxide in these mixtures will dissociate into water and oxygen. Although their stability is enhanced by HEDP, both peroxyacetic acid and peroxyoctanoic acid are also inherently unstable and will break down into acetic acid and octanoic acid, respectively. Low residual levels of these simple organic acids present on food would pose no concern. No residues of peroxyacetic acid or peroxyoctanoic acid in these mixtures were expected to remain on treated foods. Thus, the peroxide components of the peroxyacid antimicrobial mixtures did not pose toxicological concerns for the uses being considered at present and the focus of the biochemical and toxicological aspects of this safety evaluation was HEDP.

Table 1. Composition of four antimicrobial solutions (A–D) and maximum concentration of components in ready-to-use solutions (after dilution)

Component	Weight of each component in the solution at equilibrium[a] (%)				Maximum concentration of each component in the solution after dilution[b] (mg/kg)			
	A	B	C	D	A	B	C	D
Acetic acid	40.6	49.4	32.0	42.0	985	2000	208[d]	NS
Peroxyacetic acid	12	12.2	15.0	12.0	213[c]	220[c]	80	80
Hydrogen peroxide	6.2	4.5	11.1	4.0	110	150	59	59
Octanoic acid	3.2	8.8	0.0	10.0	74	300	0	NS
Peroxyoctanoic acid	0.8	1.4	0.0	3.4	14[c]	25[c]	0	NS
1-Hydroxy-ethylidene-1, 1-diphosphonic acid (HEDP)	0.6	0.6	0.9	0.6	13	13	4.8[d]	4.8
Water	36.6	23.1	41.0	28.0	—	—	—	—

NS, not stated.

[a] At equilibrium, which occurs 7–13 days after manufacture, depending on the temperature at which the solution is stored.

[b] Solutions A and B are diluted to achieve a target concentration of total peroxyacid of 200 mg/kg; to account for variations, maximum values assume that use will result in a concentration of total peroxyacids of 220 mg/kg. Solutions C and D are diluted to achieve a target concentration of total peroxyacid of 40 mg/kg; to account for variations, maximum values assume that use will result in a concentration of total peroxyacids of 80 mg/kg.

[c] Concentration of total peroxyacid as peroxyacetic acid = [220] + [(22/160) × 76]; there is variation of up to 10% in the measured concentration of peroxyacetic acid, due to differences in equipment for measurement and dispensing.

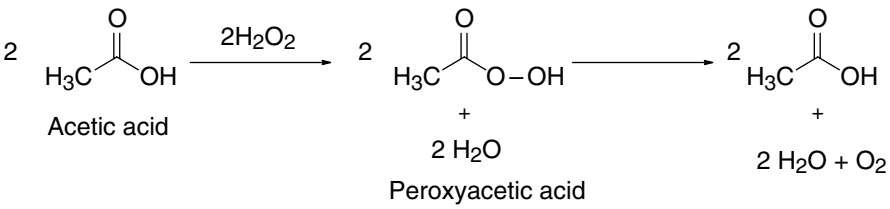
[d] Theoretical value (not based on analysis).

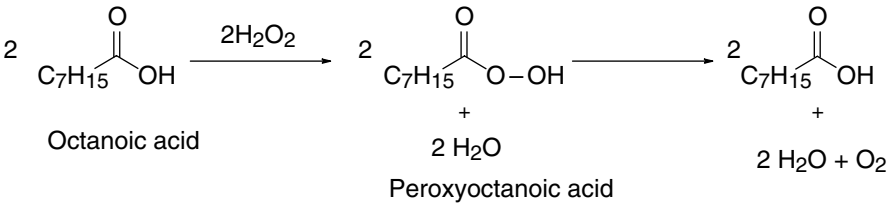
2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

Caniggia & Gennari (1977) published a concise report on the intestinal absorption and kinetics of ^{32}P-labelled EHDP (disodium ethane-1-hydroxy-1, 1 diphosphonate; disodium etidronate; referred to as HEDP in this monograph) in humans. Ten volunteers were given HEDP at an oral dose of 20 mg/kg (the carrier dose) together with 40 μCi (1480 kBq) of [^{32}P]HEDP. After 6 days, 70–90% of the administered dose was found in the faeces. Seven other subjects were given EHDP at an oral dose of 100 mg (the carrier dose) together with 20 μCi (740 kBq) of [^{32}P]HEDP intravenously. Six days after intravenous administration, 35–50% of the radiolabel administered was excreted unchanged in the urine, with negligible [^{32}P]HEDP found in the faeces. There was a rapid decline in the concentration of [^{32}P]HEDP in the plasma; after 6 days, <0.03% of the administered dose remained in the plasma. Although only limited information was published in this report, the results suggested that orally administered HEDP is poorly absorbed in humans, and that HEDP may accumulate outside of the blood.

Figure 1. Main chemical reactions in the equilibrium solutions





Hydrogen peroxide

2.1.2 Biotransformation

Michael et al. (1972) studied the absorption, distribution, and metabolism of HEDP in rats ($n = 3$ or 4), rabbits ($n = 3$), dogs ($n = 2$ or 3) and monkeys ($n = 3$) after oral administration of ^{14}C-labelled HEDP via intragastric cannula. The doses administered ranged from 10 to 50 mg/kg bw. The authors found that about 90% of the administered dose was excreted in the faeces of the adult animals. Absorption, which occurred in the stomach, was <10% in rats, rabbits and monkeys, and ranged from about 14% in older dogs to 21% in young dogs. Consistent with a possible age-dependent effect, absorption was somewhat greater in weanling rats. Some rats had been previously fed HEDP as part of their diet, but such preconditioning did not affect absorption. The authors attempted to identify metabolites in biological samples obtained from rats and dogs, but they reported that there was no metabolism of HEDP in either species. In all species, about half the absorbed dose was excreted unchanged in the urine and the rest was deposited in the bone, where its half-life in rats was demonstrated to be about 12 days. The results of these studies, conducted in a variety of species with small numbers of test animals, were consistent with the data obtained from a small sample of human volunteers. Collectively, the data indicated that absorption of HEDP from the gastrointestinal tract is very limited and its metabolism is negligible. Negligible metabolism of

systemic HEDP could have been due to the fact that carbon–phosphorus (C–P) bonds are difficult to break.

2.2 Toxicological studies

2.2.1 Acute toxicity

The available median lethal dose (LD_{50}) values for HEDP administered orally are summarized in Table 2. Two of these studies, in which high doses of HEDP were associated with kidney damage in rats and rabbits, are described more fully below.

Rats

Groups of 10 male and 10 female fasted Charles River CD rats were given HEDP (as disodium etridronate, the disodium salt of HEDP) by stomach tube, at one of four doses selected on the basis of an assumed toxicity and dose–response curve. The LD_{50} was determined to be 1.34 g/kg bw. Among the surviving animals that had received a higher dose (1.60 or 1.14 g/kg bw) of disodium etidronate, 3 out of 10 were found to have light grey, granular kidneys. Microscopic evaluation revealed damage to the nephritic tubules. The kidneys of all animals receiving the lowest dose (0.814 g/kg bw) also showed mild tubular changes. The kidney : body weight ratios of the animals at the two higher doses were significantly higher than those of animals at 0.814 g/kg bw, as well as being higher than the normal range (Nixon et al., 1972).

Rabbits

The same procedure was used to determine LD_{50} values in New Zealand rabbits. It was found that the susceptibility of rabbits to the acute effects of HEDP (as disodium etridronate, the disodium salt of HEDP) is a function of age, weight, and sex. The LD_{50} values ranged from 0.581 to 1.14 g/kg bw, and were lower in males than in females, and lower in mature animals (body weight, >3300 g) than immature animals (body weight, about 2500 g). No unusual lesions were reported upon microscopic analysis. About 50% of the surviving animals (in all groups) were found to have kidney lesions indicative of chronic interstitial nephritis; however,

Table 2. Acute toxicity of HEDP administered orally

Species	Sex	LD_{50} (g/kg bw)	Reference
Rat	Not specified	2.40	Monsanto MSDS
Rat	M, F	1.34	Nixon et al. (1972)
Rabbit	M, F	0.581–1.14	Nixon et al. (1972)
Dog	M, F	84.80[a]	Nixon et al. (1972)
Rat	M, F	3.13	Younger Laboratories (1965)

F, female; M, male.
[a] Value was estimated, due to emesis in some dogs.

chronic interstitial nephritis is commonly found in the rabbit and it is thus hard to determine whether the finding was related to treatment (Nixon et al., 1972).

Dogs

The administration of HEDP (as disodium etridronate, the disodium salt of HEDP) produced an immediate emetic response in some dogs, and it was thus not possible to clearly define an LD_{50} value. On the basis of early deaths and necropsy of animals found in a moribund condition at higher doses (1.0–10.0 g/kg), however, the LD_{50} was estimated to be about 1.0 g/kg (Nixon et al. 1972).

2.2.2 Short-term studies of toxicity

Rats

In a 91-day feeding study, groups of 20 male and 20 female Charles River CD rats were fed diets containing HEDP (disodium monohydrate salt) at 0, 0.2, 1.0, or 5.0% (equivalent to doses of 0, 100, 500, and 2500 mg/kg bw per day). Owing to severe mortality and weight loss observed at 5.0%, the study of that group was terminated after 1 week. After conclusion of the study (91 days for groups at 0, 0.2, and 1.0%, and 1 week for the 5.0% group), five males and five females from each group were randomly selected for necropsy. Histopathological lesions and/or alterations of blood parameters were observed and appeared to be associated with gastritis. At 5.0%, gastrointestinal erosions were observed and the kidney : body weight ratio was high (1.48% and 1.55% for females and males, respectively) compared with controls (1.11%). No treatment-related changes were observed in histopathological lesions or blood haematological values at 0.2% or 1.0%. The kidney : body weight ratio in females at 1.0% was slightly higher (at 0.82%) than controls (0.64%). All other parameters measured in the study were normal and similar to those in the controls. Hence, the no-observed-effect level (NOEL) was 1.0%, equivalent to a dose of 500 mg/kg bw per day (Nixon et al., 1972).

A 90-day feeding study in rats was designed to assess the toxicity of HEDP (crystalline sodium salt characteristic of DEQUEST®2010 phosphonate in the nature and amount of by-products and impurities). Groups of 15 male and 15 female rats were given HEDP at a dietary concentration of 0, 3000, 10 000 or 30 000 mg/kg of feed (equivalent to 0, 150, 500, or 1500 mg/kg bw per day) of HEDP. Body weights, food consumption and mortality were determined weekly. At 45 and 90 days, haematology and clinical chemistry parameters were assessed and urine analysis was performed. The animals were necropsied at the end of the study and histopathological examinations were performed on tissues from animals treated at the highest dose only. A high level of mortality was observed at 30 000 mg/kg. This finding might be related to the ingestion of HEDP, although it was possibly the result of trauma induced by blood collection. At 30 000 mg/kg, body-weight gain was inhibited in males. At the highest dose tested, haematology revealed significant changes, including increased erythrocyte counts in males, decreased haemoglobin concentration and erythrocyte volume fractions in males and females, and decreased leukocyte counts at the end of the study in females only. The lesions observed in histopathological examinations, which were con-

ducted on animals treated at the highest dose only, were described by the pathologist as being typical of the controls. At 10 000 mg/kg (500 mg/kg bw per day), no adverse effects were noted in any parameters measured in this study; however, histopathological examinations were not conducted on rats in the groups given the two lower doses. The NOEL was 500 mg/kg bw per day (Industrial Bio-Test Laboratories, Inc., 1975a).

Dogs

In a 90-day study of toxicity designed to test the effects of HEDP (crystalline sodium salt characteristic of DEQUEST® 2010 phosphonate in nature and amount of by-products and impurities), groups of four male and four female beagle dogs (aged 5 months at the start of the study) were given HEDP at a concentration of 0, 1000, 3000, or 10 000 mg/kg of diet (equivalent to a dose of 0, 25, 75, or 250 mg/kg bw per day). Food and water were available ad libitum. Body weights and food consumption were recorded weekly. Haematology and blood chemistry parameters were assessed and urine analysis was conducted at the beginning of the study and at 56 and 85 days. At the end of the study, organ weights were determined and gross and histopathological examinations were conducted. There were no adverse effects of the test material on body weight, although food intake in females at the intermediate and highest doses was decreased compared with that of the controls. No deaths were reported. Small changes in haematological parameters (increased erythrocyte counts and decreased mean corpuscular volume) and variations in blood chemical parameters (serum potassium and magnesium concentrations in males and females, respectively) were noted, but the effects were inconsistent and were not attributed to treatment. Increased numbers of leukocytes and crystals were found in the urine of dogs from all treatment groups at the final analysis. However, this was not considered to be a significant finding since no changes in the genitourinary system were observed on microscopy. Some differences were noted in organ weights, including increased brain weights in females at the intermediate and highest doses and increased thyroid weights in males at the highest dose. These differences, which were not associated with microscopic changes in these organs, were not considered to be related to treatment. There were no gross or histopathological changes reported for any of the tissues or organs examined in this study. The NOEL was 250 mg/kg bw per day (Industrial Bio-Test Laboratories, Inc., 1975b).

2.2.3 Long-term studies of toxicity and carcinogenicity

Long-term studies to address the toxicity or carcinogenic potential of HEDP were not available for this evaluation. The publication by Nixon et al. (1972) refers to data obtained in chronic tests that were “to be published elsewhere”. However, a literature search failed to reveal any data resulting from traditional long-term tests of toxicity in animals. One study on the skeletal effects of HEDP administered subcutaneously to beagle dogs for approximately 1 or 2 years is described below (see 2.2.6, special study).

2.2.4 Genotoxicity

The potential genotoxicity of HEDP was assessed using an assay for reverse mutation in which five strains of *S. typhimurium* (TA98, TA100, TA1535, TA1537 and TA1538), were tested, with and without metabolic activation provided by rat liver microsomes, at doses of 0.001, 0.01, 0.1, 1, 5 or 10 µl/plate (in water). The test article was a commercial product that contained 60% HEDP in aqueous solution. Aberrations in the background lawn were observed at concentrations of 5 and 10 µl/plate, indicating toxicity at the two highest concentrations tested for all five strains of *Salmonella*. The tester strains responded as expected to solvent and positive controls. Under the conditions of the assay, the test article was not mutagenic (Monsanto Co., 1977).

The potential genotoxicity of HEDP was also assessed by thymidine kinase (*Tk*) gene forward mutation assay in mouse lymphoma L5178Y cells, with and without metabolic activation provided by rat liver microsomes; at doses of 0.064, 0.125, 0.250, 0.500, or 0.600 µl/ml in the absence of microsomal enzymes and 0.125, 0.250, 0.500, 0.600 and 0.800 µl/ml in the presence of microsomal enzymes. The test article, a commercial product that contained 60% HEDP in an aqueous solution, was the same as that tested in the assay for reverse mutation, but was in this case diluted in dimethyl sulfoxide (DMSO). At concentrations of ≥0.5 µl/ml, cytotoxicity was observed that was greater in the presence of microsomal enzymes. In the first of the two trials, although control values for spontaneous mutagenesis were higher than expected, the incidence of mutagenesis caused by HEDP at the highest concentration tested was more than 2.5 times that for the controls for spontaneous mutagenesis with microsomal activation. In the second trial, the incidence of spontaneous mutagenesis was not elevated and the incidence of mutagenesis caused by HEDP at the highest concentration tested was about twice that for the controls for spontaneous mutagenesis. The positive controls gave the expected results. Under the conditions of the assay, the test article did not induce forward mutation in the mouse lymphoma assay (Litton Bionetics, Inc., 1978).

2.2.5 Reproductive toxicity

Rats

In a combined two-generation study of reproductive toxicity and teratogenicity, five groups of 22 female and 22 male weanling Charles-River rats were given the disodium salt of HEDP (disodium etidronate) at a dietary concentration of 0, 0.1 or 0.5% (equivalent to a dose of 0, 50 or 250 mg/kg bw per day), either continuously or only on days 6–15 of gestation for two generations. Reproductive endpoints and offspring parameters were analysed in the F_{1a}, F_{1b}, and F_{2a} litters. The third litter of the F_1 generation (F_{1c}) and the second litter of the second generation (F_{2b}) were used in teratological examinations. During the teratology phase, half of the animals in each group were sacrificed at day 13 and the others at day 21 of gestation. Body-weight gains were similar for all groups in both generations, and the overall conception rate was 90%, indicating that the compound did not interfere with spermatogenesis or ovulation. In the first generation, at the highest dose, the number of pubs born in the first litter (F_{1a}) was reduced and there was an increase

in stillborn pups in the second litter (F_{1b}). The rate of mortality in pups after birth was low and the weights of pups at day 4 and at weaning were the same. Teratological examination of the third litter (F_{1c}) showed no differences in resorptions or implantations in females sacrificed at day 13 (corpora lutea were not counted) and no differences in live fetuses, corpora lutea, or implantation at 21 days. At day 21, however, significant resorptions were reported in the controls. In the second generation, the first litters (F_{2a}) were smaller than the litters in the first generation, but there were no other differences in reproductive parameters. During the teratology phase, no differences in corpora lutea, implantations, or resorptions were noted in rats sacrificed at 13 days. In continually fed rats sacrificed at 21 days, the number of implantations was reduced and corpora lutea formation was depressed at the highest dose. A decrease in the number of live fetuses at the highest dose, significant only in rats fed during gestation, was also observed. The incidence of defective pups was similar to that in control animals and the study authors concluded that disodium etidronate was not teratogenic in rats at either dose tested. The NOEL, based on reduced litter size and decreased number of pups at the highest dose, was 50 mg/kg bw per day (Nolen & Buehler, 1971).

Rabbits

In a combined study of reproductive toxicity and teratogenicity in rabbits, two separate experiments were conducted. In the first experiment, four groups of 25 virgin New Zealand white rabbits were given HEDP (as an aqueous solution of disodium etidronate) at a dose of 0, 100, 250 or 500 mg/kg bw per day via intubation on days 2–16 of gestation. The rabbits were inseminated (day 1) and dosing commenced before implantation (day 7). After 4–5 consecutive doses of HEDP at 500 mg/kg bw per day, the pregnant rabbits died. Four rabbits survived three daily doses of HEDP at 500 mg/kg bw per day and these animals subsequently received HEDP at 250 mg/kg for the rest of the study. At a dose of 100 mg/kg bw per day, HEDP caused a 68% reduction in the conception rate in rabbits. Owing to toxicity at 500 mg/kg bw per day and a reduced conception rate at the lowest dose tested (i.e.100 mg/kg bw per day), a second experiment was performed in which the highest dose tested was 100 mg/kg bw per day.

In the second experiment, four groups of 25 virgin New Zealand white rabbits were given HEDP (disodium etidronate) at a dose of 0 (water), 25, 50, or 100 mg/kg bw per day in the diet or 100 mg/kg by gavage, on days 2–16 of gestation. An additional untreated control group was also used in this study. Reproductive parameters and offspring malformations were analysed. The authors reported no statistical differences in food consumption or body-weight gain, although these data were not presented. However, the authors noted that rabbits given the highest dose (100 mg/kg bw per day) consumed the least amount of food and gained the least weight. The conception rate in dams given disodium etidronate at a dose of 100 mg/kg bw per day by gavage or in the diet was 90% or 95%, respectively, indicating no effect on conception or nidation. No differences were observed in the numbers of corpora lutea, resorptions, or live fetuses. Fetuses from dams given disodium etidronate at a dose of 100 mg/kg bw per day by gavage were significantly smaller than those from untreated controls. No differences in the defective

fetuses in treated groups compared with the controls were reported. Very few skeletal defects were seen, although variations in the number of ribs and sternebrae occurred in up to 50% of the rabbit fetuses. The study authors stated that these variations in the ribs and sternebrae were not teratogenic effects (Cozens, 1965). They thus concluded that there were no treatment-related adverse effects on reproduction parameters and that disodium etidronate is not teratogenic in rabbits (Nolen & Buehler, 1971).

Nolan & Buehler (1971) speculated that the effects on conception rate in rabbits given HEDP (disodium etidronate) at a dose of 100 mg/kg per day in their first experiment may have resulted from stress caused by gavage, because a reduction in conception rate was not observed in their second experiment. In the second experiment, however, the authors reported a reduction in fetal weights with HEDP at a dose of 100 mg/kg bw per day administered by gavage, which they attributed to slightly larger litters. Although, not statistically significant, decreases in food consumption and body-weight gain in dams at the same dose were reported in the second experiment. On the basis of decreased fetal weights, the NOEL was set conservatively at 50 mg/kg bw per day (Nolan & Buehler, 1971).

2.2.6 Special study: skeletal effects in dogs

In a long-term study to determine the skeletal effects of HEDP, adult female beagle dogs (aged 3–4 years at the start of the study) were given HEDP at a dose of 0, 0.1, 0.5, 2, 5 or 10 mg/kg bw per day via subcutaneous injection for different times ranging from 1 to 2 years. There were 10 dogs in the control group and five dogs in each treatment group. Dogs at the two lower doses (0.1 and 0.5 mg/kg bw per day) were treated for 2 years. Dogs at 5 mg/kg bw per day were sacrificed after 13.5 months, while dogs at 2 and 10 mg/kg bw per day were sacrificed after 12 months. At the two lower doses, there was a slight reduction in osteoblastic activity, reduction in the percentage of bone surfaces with active mineralization, a reduction in mineralization rates, and a reduction in resorption spaces, but no change in osteoid seam width. There were no treatment-related fractures at 0.1 mg/kg bw, but radiographic studies indicated that the incidence of fractures was slightly increased at 0.5 mg/kg bw per day. Profound effects on bone parameters were observed at doses of 2–10 mg/kg bw per day. The number of resorption spaces was reduced and mineralization activity was blocked to the extent that osteoid seams became thickened. At these higher doses, the incidence of fractures was markedly increased and fractures were radiologically apparent after 9–12 months. Healing of fractures, when they occurred, was inhibited at doses of HEDP of >0.5 mg/kg bw per day. The authors suggested that high doses of HEDP did not cause any permanent change in the skeleton that would interfere with fracture healing. This study indicates that HEDP caused profound effects on the skeletal system that are dose-related and dependent on the period of treatment, but that the effects are reversible (Flora et al., 1981).

Flora et al. (1981) also pointed out that oral administration of the disodium salt of HEDP at a dose of 5 mg/kg bw per day for up to 6 months is recommended for the treatment of Paget disease in humans. The authors indicated that the dose of HEDP that resulted in the development of spontaneous fractures in dogs was

about 10 times higher than the dose recommended for extended use in humans. This is based on the assumption that gastrointestinal absorption of orally administered HEDP would occur at a rate of 1% in humans. Thus, a orally administered dose of HEDP of 5 mg/kg bw per day would be expected to lead to a systemic dose of 0.05 mg/kg bw per day in humans, or 3 mg/day for an adult with a body weight of 60 kg.

2.3 Environmental studies

HEDP can also undergo photolysis to acetate and phosphate within a few days (Steber & Wierich, 1986). In distilled water and in the presence of calcium, no photodegradation of HEDP was observed, but the addition of Fe(III) and Cu(II) resulted in rapid photodegradation (Fischer, 1993). Thus, after the use of the antimicrobial solutions, residual HEDP in foods may undergo photolysis before the treated foods are consumed.

2.4 Microbiological aspects

2.4.1 Role of components in antimicrobial solutions

Different antimicrobial wash solutions are added to water to spray, wash, rinse, dip, cool or otherwise process meat, poultry, and fresh as well as processed fruits and vegetables. The solutions are used to inhibit the growth of *Salmonella* sp., *Campylobacter jejuni*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7, and spoilage and decay organisms on the product or surface to be treated (Table 3).

Peroxyacetic acid (also referred to as peracetic acid) is the major active ingredient in all of the antimicrobial wash solutions. The effect of peroxyacetic acid is

Table 3. The intended uses of four antimicrobial wash solutions[a]

Solution	Product/surface to be treated	Function
A	Poultry carcasses, parts, and organs	Antimicrobial efficacy against *Salmonella* sp., *C. jejuni*, *L. monocytogenes*, *E. coli* O157 : H7 and spoilage organisms on poultry
B	Meat carcasses, parts, trims, and organs	Antimicrobial efficacy against *Salmonella sp.*, *L. monocytogenes*, *E. a coli O157 : H7* and spoilage organisms on meat
C	Post-harvest, fresh-cut, and further processed fruits and vegetables, including process water	Antimicrobial efficacy against spoilage and decay organisms on treated fruits and vegetables and in process water
D	Further processed fruits and vegetables	Antimicrobial efficacy against S*. a javiana*, *L. monocytogenes*, *E. coli O157 : H7*, spoilage and decay organisms on further processed fruit and vegetable surfaces.

[a] See Table 1 for the composition of these solutions.

similar to that of other antimicrobial agents that function as oxidizing agents, and which attack multiple cell sites and can disrupt the chemiosmotic balance. A recently published summary (Kitis, 2004) stated that peracetic acid was identified to have antimicrobial properties as early as 1902; that it had often been used in 'cold sterilization' procedures for medical instruments and had been found to be bactericidal at 0.001%, fungicidal at 0.003% and sporicidal at 0.3%; and that it had been used in the production of gnotobiotic (germ-free) animals. This publication also proposed that the antimicrobial action of peracetic acid may result from the oxidation of proteins and, in particular, their sulfhydryl bonds. Alternatively, peracetic acid may disrupt the chemosmotic functions of outer membrane lipoproteins and oxidize nitrogenous bases in DNA, resulting in cell death. Peracetic acid was compared favourably with chlorine-based compounds; it was proposed that its antimicrobial efficacy was similar and its decomposition to the environmentally safe products acetic acid, water and oxygen provides an advantage over chlorine-based products.

Octanoic acid also contributes to the efficacy of these antimicrobial solutions. A publication by Sun et al. (2002) concludes that at lower pH, caproate (C6:0) and caprylic acid (C8:0, the alternative name for octanoic acid) inhibit microbial growth. In addition, octanoic acid functions as a surfactant to aid in wetting hydrophobic surfaces, particularly on meat.

While acetic acid and hydrogen peroxide are known to have antimicrobial effects, their effects within these solutions are minimal. Acetic acid and hydrogen peroxide are, however, in equilibrium with the peroxyacetic acid, so their presence is critical for the antimicrobial effects of the peroxyacetic acid. Peroxyoctanoic acid does not have antimicrobial activity. It is present in the solution because it is produced when octanoic acid reacts with hydrogen peroxide. HEDP has no antimicrobial effects. It functions as a stabilizer in these solutions by preventing metal ions from catalysing the breakdown of peroxyacetic acid and hydrogen peroxide.

2.4.2 Studies of antimicrobial efficacy

Laboratory and in-plant studies were done on four antimicrobial wash solutions, described as solutions A, B, C and D in Tables 1 and 3, to demonstrate the reduction of microbes for the intended use of each solution. Overall, the results of these tests indicate modest reductions in the number of surface microbes on poultry and meat. In wash water for fresh and processed fruits and vegetables, greater reductions in concentrations of microbes were observed. The results of studies that were available for this evaluation are described below.

(a) Solution A

The proposed use of antimicrobial wash solution A is for addition to water used for spraying or submerging, or spraying followed by submerging eviscerated poultry carcasses. Tests were done to compare specimens treated with water with those treated with the test substance. Thus, the key result is the net reduction beyond that found with water only. There were three groups, a group that was submersion-chilled, a group that was sprayed, and a group that was sprayed, then

submerged. The mean $\log_{10}$ reductions using United States Department of Agriculture procedures for carcass processing are listed in Table 4. These results indicate that a modest net reduction of up to about $\log_{10}$ 0.8 can be achieved from these treatments (unpublished data from the submitter).

A second set of tests was performed on pathogens (*Listeria monocytogenes*, *Salmonella typhimurium*, and *Escherichia coli* O157:H7) on different chicken parts (carcasses, wings, and livers). Net $\log_{10}$ reductions in pathogens varied from a modest to a considerable amount (from $\log_{10}$ 0.32 to 0.75 for *S. typhimurium*, from $\log_{10}$ 1.13 to 2.11 for *L. monocytogenes*, and from $\log_{10}$ 0.82 to 3.17 for *E. coli* O157:H7).

(b) Solution B

The proposed use of antimicrobial wash solution B is for adding to water used for spraying beef carcasses. The solution was diluted appropriately and added to water for spraying beef. Three separate test runs were conducted. In the first test, 10 randomly selected carcasses were selected; in the second test, 29–30 randomly selected carcasses were selected, and in the third, 128 carcasses were selected in-plant. In all tests, the carcasses were aseptically sampled by tissue excision, either before treatment, after treatment, or at final inspection, then serially diluted and plated. The number of colonies formed (CFU/cm^2) for all aerobic bacteria (total aerobic plate counts), coliforms, and *E. coli* were determined. For these trials, reductions ranged from $\log_{10}$ 0.434 (standard deviation (SD), 1.083) to 1.05 (SD, 0.495) for samples taken immediately after treatment and from $\log_{10}$ 0.246 (SD, 1.221) to 0.573 (SD, 0.567) at the final inspection. In essence, the values indicated that a modest, but highly variable, initial reduction of microorganisms was followed by some renewed microbial growth or acquisition of more microbes.

When pathogens were inoculated onto beef, reductions in the numbers of microbes were modest, approximately $\log_{10}$ 0.5 to 1.0 more than reductions after washing with water only. The specific results are summarized in Table 5. The relative reductions reported were modest, ranging from $\log_{10}$ 0.5 to 1.3 (unpublished data from the submitter).

Microbial contamination primarily occurs on the surface of meats. Various spraying and dipping methods, usually transient in nature, are employed to remove surface bacteria. Although several chemicals are employed in these methods, the levels of reduction of microbes, with respect to resident bacteria and specific pathogens, are typically low. In a recent publication, the use of one of these products was compared with other methods (Gill & Badoni, 2004); the results indicated that use of a solution containing 0.02% peroxyacetic acid was associated with modest reductions in the number of pathogens from $\log_{10}$ 0.5 to 1.0 l compared with meat treated with water only, but reductions after treatment with lactic acid were $\log_{10} > 1$.

Table 4. Mean log_{10} reductions of microbes on poultry carcasses treated with water or with antimicrobial wash solution A

Poultry process	Aerobic plate counts (log_{10} reduction)			*E. coli* (log_{10} reduction)			Coliforms (log_{10} reduction)		
	Water	Solution A	Net reduction	Water	Solution A	Net reduction	Water	Solution A	Net reduction
Submerged	0.53	1.21	0.68	0.56	1.37	0.81	0.6	1.27	0.67
Sprayed only	0.46	0.62	0.16	0.46	0.84	0.38	0.33	0.64	0.31
Submerged then sprayed	0.84	1.33	0.49	0.85	1.44	0.59	0.78	1.31	0.53

From unpublished data from the submitter.

Table 5. Mean log_{10} reductions in specific pathogens inoculated onto beef washed with water or with antimicrobial wash solution B

Pathogen	Average log_{10} reduction		Relative log_{10} reduction, solution B relative to water
	Water	Solution B	
L. monocytogenes	0.7	1.22	0.52
S. typhimurium	0.32	1.62	1.3
E. coli	0.4	1.48	1.08

From unpublished data from the submitter.

Table 6. Mean log_{10} reductions in microorganisms found in water treated with antimicrobial wash solution C relative to untreated water

Residual peroxyacetic acid (mg/kg)	Log_{10} reduction (log_{10}CFU)
<3	≤2
10–30	2–4
40–50	5–6

From unpublished data from the submitter.
CFU, colony-forming units.

(c) Solution C

The proposed use of antimicrobial wash solution C is for addition to water used for processing vegetables for post-harvest, fresh-cut, and further processed fruit and vegetables. Peroxyacetic acid at a concentration of 10–30 mg/kg was added to water for processing vegetables. There was a reduction of up to 4-log (log_{10} 4) in the relative concentrations of microorganisms found in the treated wash water, compared with the untreated wash water; this correlated with the amount of residual peroxyacetic acid (Table 6) (unpublished data from the submitter).

(d) Solution D

The proposed use of antimicrobial wash solution D is for reduction of contamination, either for organisms on surfaces or for cross-contamination in wash water, on the surface of processed fruit and vegetables. To test the reduction of contamination, tomato surfaces were inoculated with *E. coli O*157:H7, *L. monocytogenes*, and *S. javiana* and treated with either tap water or Tsunami 200. The results indicated that solution D effectively reduced numbers of these pathogens (Table 7).

To test for the elimination of cross-contamination, cherry tomatoes were inoculated with the same target pathogens, which were allowed to attach for 24 h. Inoculated and non-inoculated cherry tomatoes were then submerged in solution D (Tsunami 200) or in tap water. The non-inoculated tomatoes were removed to a

Table 7. Mean log_{10} reductions in pathogens on tomato surfaces treated with water only or with antimicrobial wash solution D

Pathogen	Pathogens on tomato surfaces (log_{10} CFU)		
	Water	Solution D	Log_{10} reduction
L. monocytogenes	4.73	0.00	4.73
E. coli	5.00	0.87	4.13
S. javiana	2.62	0.00	2.62

From unpublished data from the submitter.

neutralizing solution, which was vortexed to remove bacteria from the tomatoes, then diluted and plated. There was a reduction of greater than 2-log (log_{10} 2) in all three pathogens transferred by cross-contamination (unpublished data from the submitter).

2.5 Observations in humans

The disodium salt of HEDP, which is known clinically as sodium etidronate, is used to treat Paget disease, which is an idiopathic disease characterized by accelerated bone metabolism. Fractures and other abnormalities of bone are common in patients with Paget disease. Due to its high affinity for solid-phase calcium phosphate, HEDP prevents hydroxyapatite crystal growth and dissolution on crystal surfaces of bone. Its mechanism of action, however, is not fully understood.

The recommended dose of sodium etidronate is 5–10 mg/kg bw given orally once daily for 6 months or less, or 11–20 mg/kg bw per day for 3 months or less. Doses in excess of 20 mg/kg bw per day are not recommended. The dose must be reduced in cases of renal insufficiency. Sodium etidronate is generally well tolerated and the incidence of side-effects is low (Center for Drug Evaluation and Research, 2001; Physician's Desk Reference, 2004). Initial therapy with a dose of 5 mg/kg bw per day of sodium etidronate appears to maximize benefits for patients with Paget disease while minimizing possible adverse effects (Canfield et al., 1977).

Numerous abstracts/citations addressing the use of HEDP in cancer therapy, osteoporosis, nuclear imaging, and hypercalcaemia associated with malignancy, and other disorders of calcium and phosphorus balance have been published. Such studies were not considered to be relevant to food safety and are beyond the scope of this assessment.

3. INTAKE

3.1 Residues on foods

The use of the four solutions of peroxyacid in antimicrobial water washes for the processing of meat, poultry, fruits, and vegetables results in predictable

residues on treated foods. The hydrogen peroxide in the solution and the peroxyacetic and peroxyoctanoic acids formed in situ are inherently unstable, especially in the presence of oxidizable organic material. Therefore, there would be no expected residues of these substances on treated foods. Acetic and octanoic acid present in the solution 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, as would HEDP, which is stable and non-reactive under the conditions of use.

Acetic and octanoic acids are components of many foods and are also used as flavouring agents in foods. The Committee has previously evaluated both of these substances. The minor residues of these substances remaining on treated foods result in exposures that are insignificant in comparison to those from consumption of foods containing the substances naturally, or as added flavouring agents. 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. A highly conservative estimate of exposure for octanoic acid of 1.9 mg per day resulting from the use of the antimicrobial solutions was noted by the Committee. This estimate was prepared employing WHO Global Environment Monitoring System — Food Contamination Monitoring and Assessment Programme (GEMS/Food) international diets. Intake of acetic acid was not explicitly analysed, but its use in and on foods (vinegar) would result in a greater food exposure than that from octanoic acid. Exposure to these common food acids was not further considered in this evaluation.

HEDP is expected to remain on foods that are treated with the antimicrobial washes and not further washed, processed, or cooked. The Committee considered submitted information concerning residues of HEDP on foods. Studies were conducted with meats, poultry, fruits, and vegetables, each treated with one of the solutions under typical conditions of use. The foods were allowed to drain, but were not further processed or cooked. It was assumed that all additional weight in the meats treated was attributable to the antimicrobial wash; the concentration of HEDP in the solutions was used to determine the residual concentration of HEDP present in the meat. Poultry was further treated to recover any HEDP present. Fruit and vegetables were washed with deionized water to recover the residual HEDP. For vegetables, lower- and upper-bound estimates of intake were made based on the differing surface areas of the treated foods. Broccoli, a vegetable with a high surface area, provided the data for the upper-bound estimates, while tomato was used to provide the lower-bound estimates. Furthermore, for processed fruit and vegetables, it was assumed that each would be treated twice; before cutting or processing and again afterwards. Thus, the measured residues were doubled, assuming no loss from either treatment. The results are reported in Table 8 (unpublished data from the submitter).

3.2 International estimates of intake

The Committee considered international estimates of intake of HEDP, prepared using food information taken from the GEMS/Food regional diets and the data on HEDP residues from Table 8. The intake of every food that could be treated with

Table 8. Residues of HEDP in treated foods

Type of food treated	Residue of HEDP (μg/kg, ppb)
Meats	
Carcasses	58
Parts/trim	161
Poultry	198
Fruit and vegetables (single treatment)	
Low surface area	4.2
High surface area	67.5
Fruits and vegetables (double treatment)	
Low surface area	8.4
High surface area	135

From unpublished data from the submitter.

HEDP was combined with the appropriate residue concentration for each of the five regional diets. Two estimates were prepared for each region; one using the residue concentration for a vegetable with a low surface area and the other using the residue concentration for a vegetable with a high surface area. It was assumed that there would be no reduction in HEDP residues after washing or cooking. Further, it was assumed that all fruit and vegetables would be treated three times with the antimicrobial solution with no loss; once on the raw commodity and twice during further processing.

The highest estimate of intake was from the European diet; 3.6 μg/kg bw per day for the upper-bound estimate using a model for a vegetable with a high surface area. All the estimates are summarized in Tables 9 and 10.

3.3 National estimates of intake

The Committee considered three national estimates of intake. The first was a total diet study from the Czech Republic. The two remaining studies were based on individual dietary records in the USA and the UK, respectively.

Completed in 1995, the Czech total diet study considered 160 foodstuffs. The foods were prepared using standard recipes. Each food that might be treated with the antimicrobial solution was considered, with a food intake matched to an HEDP concentration from Table 8. Lower-bound and upper-bound estimates were made using the data for vegetables with low surface area and data for the vegetables with high surface area separately. Average daily food consumption values for the Czech Republic were used. The lower-bound estimate of exposure was 0.405 μg/kg bw per day and the upper-bound estimate was 2.224 μg/kg bw per day.

The estimates from the USA and the UK were made in a similar manner. For each individual surveyed, all foods that could have been treated with the antimicrobial solution were considered. The appropriate concentration of HEDP residue was multiplied by the intake of each food and the total intake of HEDP for each

Table 9. International estimates of intake of HEDP (lower bound)

GEMS/ Food code	Food	HEDP residue (μg/kg, ppb)	Intake of HEDP (μg/kg bw per day) in GEMS/ Food regional diet				
			Middle East	Far East	Africa	Latin America	Europe
VR75	Roots	12.6	0.013	0.023	0.067	0.033	0.051
VD70	Pulses	12.6	0.005	0.004	0.004	0.005	0.003
VD70	Nuts	12.6	0.003	0.011	0.007	0.012	0.006
VD70	Vegetable fat	12.6	0.008	0.003	0.005	0.005	0.008
HS93	Spices	12.6	0.001	0.001	0.000	0.000	0.000
HS93	Vegetables	12.6	0.049	0.038	0.016	0.032	0.078
PE112	Fruit	12.6	0.043	0.018	0.020	0.057	0.045
MO105	Offal	68	0.005	0.002	0.003	0.007	0.014
MO105	Meat	68	0.042	0.037	0.027	0.053	0.176
PM110	Poultry	198	0.102	0.044	0.018	0.083	0.175
PO111	Poultry offal	198	0.000	0.000	0.000	0.001	0.001
PF111	Poultry fat	198	0.010	0.004	0.002	0.008	0.017
MF95	Mammalian fat	68	0.001	0.002	0.001	0.005	0.009
Total intake			0.321	0.222	0.114	0.211	0.753

Table 10. International estimates of intake of HEDP (upper bound)

GEMS/ Food code	Food	HEDP residue (μg/kg, ppb)	Intake of HEDP (μg/kg bw per day) in GEMS/ Food regional diet				
			Middle East	Far East	Africa	Latin America	Europe
VR75	Roots	202.4	0.208	0.366	1.084	0.537	0.816
VD70	Pulses	202.4	0.083	0.067	0.060	0.078	0.041
VD70	Nuts	202.4	0.043	0.169	0.115	0.194	0.101
VD70	Vegetable fat	202.4	0.136	0.048	0.079	0.074	0.130
HS93	Spices	202.4	0.008	0.010	0.006	0.002	0.002
HS93	Vegetables	202.4	0.786	0.604	0.260	0.508	1.254
PE112	Fruit	202.4	0.689	0.288	0.319	0.915	0.716
MO105	Offal	68	0.005	0.002	0.003	0.007	0.014
MO105	Meat	68	0.042	0.037	0.027	0.053	0.176
PM110	Poultry	198	0.102	0.044	0.018	0.083	0.175
PO111	Poultry offal	198	0.000	0.000	0.000	0.001	0.001
PF111	Poultry fat	198	0.010	0.004	0.002	0.008	0.017
MF95	Mammalian fat	68	0.001	0.002	0.001	0.005	0.009
Total intake			2.153	1.676	1.994	2.515	3.623

Table 11. Estimates of intakes of HEDP used in antimicrobial wash solutions

Estimates	Exposure (μg/kg bw per day)
GEMS/Food	3.6 (European regional diet)
Czech Total Diet Study	2.224 (mean)
USA dietary records	4.706 (90th percentile, upper bound)
UK dietary records	3.263 (90th percentile, upper bound)

food was calculated for each individual. The mean and 90th-percentile intakes for the whole population were computed from the individual records. The data on food intake from the USA were taken from the USA Department of Agriculture Continuing Survey of Food Intakes by Individuals, 1994–6, 1998. The data on food intake from the UK were taken from the Ministry of Agriculture, Food, and Fisheries Dietary and Nutritional Survey of British Adults, 1986–7. Here again, lower-bound and upper-bound estimates were made using the data for vegetables with low surface area and vegetables with high surface area separately.

The mean estimate of intake for the USA was 0.357 (lower bound) or 2.235 (upper bound) μg/kg bw per day. The corresponding intakes for individuals at the 90th percentile of consumption were 0.740 and 4.706 μg/kg bw per day, respectively. The mean estimate of intake for the UK was 0.243 (lower bound) or 1.795 (upper bound) μg/kg bw per day. The corresponding intakes for individuals at the 90th percentile of consumption were 0.458 and 3.263 μg/kg bw per day, respectively.

Table 11 summarizes the estimates of intake of HEDP used in antimicrobial wash solutions.

3.4 Non-food uses of HEDP

HEDP is used as an anti-scaling agent for water treatment and in boilers. The regulatory limit for this use in the USA is 25 μg/l. However, HEDP is known to be used in the rest of the world, including China. It is also used as a drug for treatment of Paget disease (a disease of excessive bone turnover) and in some over-the-counter cosmetic and pharmaceutical formulations. The Environmental Protection Agency in the USA has estimated exposure to HEDP from these uses to be no more than 6 μg/kg bw per day, including 0.04 μg/kg bw per day from its use on food (Environmental Protection Agency, 1998). The Committee noted that this estimate of exposure for food uses of HEDP was much less conservative than that evaluated herein, assuming that cooking and further processing of treated foods would result in concentrations of HEDP of no greater than 1 μg/kg on food as consumed.

4. STUDIES ON THE QUALITY, NUTRITIONAL VALUE, OR OTHER PROPERTIES OF FOOD TREATED WITH ANTIMICROBIAL SOLUTIONS

4.1 Thiobarbituric acid and fatty acid profiles of meat and poultry products

The antimicrobial wash solution identified as solution A was added to water used for spraying and dipping poultry carcasses in a study to determine whether the treatment resulted in significant differences in thiobarbituric acid (TBA) and fatty acid profiles of raw or cooked poultry products. No differences were found when compared with treatment with water (Ecolab, Inc., 2000).

Samples of fresh beef were exposed to antimicrobial wash solution B, which contains total peroxyacids at a concentration of 200 mg/kg, to determine whether the treatment resulted in significant differences in TBA and fatty acid profiles of cooked and uncooked meat (Ecolab, Inc., 1999a, 1999b). Cooking to an internal temperature of 175 °F increased the TBA value by eightfold relative to uncooked samples. No differences in TBA or fatty acid profiles compared with treatment with water were found. There was a slight difference ($p = 0.54$) in values for myristic acid between raw meat and cooked meat treated with peroxyacid; this was attributed to cooking or variation in the meat samples tested.

The reagent TBA is commonly used to determine the extent to which animal and vegetable fats and oils (including fatty acids, their esters, and related substances) are oxidized. Thus TBA values provide a measure of rancidicity. The results of testing for TBA and the determinations of fatty acid profiles described above suggested that treating poultry or meat with solutions A or B did not adversely impact the quality of treated poultry or meat products, respectively.

4.2 The effect of the potential reactivity of hydrogen peroxide and peroxyacetic acid on meat and poultry products

In a study by Upendraroa et al. (1972), vegetable oils placed in contact with 30% or 60% hydrogen peroxide and 5% or 17% peroxyacetic acid for 2–10 h underwent epoxidation. Other studies that used high concentrations of hydrogen peroxide and long periods of contact were found in the literature and mainly indicated potential reactions of hydrogen peroxide with other food components. The low concentrations of the components of antimicrobial solutions in ready-to-use wash solutions and sprays, and the transient nature of their contact with food is expected to prevent potential oxidation reactions from occurring on food. The low reactivity potential of solutions C and D has been confirmed in a study of their effects, under the intended condition of use, on fruit and vegetables (Ecolab, Inc., 1995).

4.3 Nutritional tests to determine the effects of peroxyacetic acid and hydrogen peroxide on fruit and vegetables

A study was conducted to determine the effects of peroxyacetic acid and hydrogen peroxide on the nutrient content of fruit and vegetables (Ecolab, Inc.,

1995). Tomatoes, potatoes, and broccoli were prepared for consumption, exposed to solution C (containing peroxyacetic acid at 80 mg/kg and hydrogen peroxide at 59 mg/kg) for 5 min ('worse-case' conditions of exposure), then rinsed. Control and treated samples were analysed for effects on β-carotene and vitamin C, nutrients chosen for analysis because of their susceptibility to oxidation and other degradation reactions. There was no effect on β-carotene content in tomatoes or broccoli. There was no effect on vitamin C in potatoes or broccoli. There was a treatment-related decrease of 37% in the ascorbic acid content of tomatoes, which occurred in conjunction with an equivalent increase in dehydroascorbic acid content. Thus, the active content of vitamin C (Sabry et al., 1958) in tomatoes was unchanged (unpublished data from the Pillsbury Company). These results indicated that the use of antimicrobial wash solution C on fresh fruits and vegetables would not be expected to adversely affect their nutrient content.

5. COMMENTS

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 break down 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 mouse lymphoma L51718 $Tk^{+/-}$ 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 and 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 per day 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 might not be long enough to observe skeletal effects in dogs and that there might 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.

Antimicrobial efficacy

Information available to the Committee indicated that solutions of peroxyacetic acid 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 *L. monocytogenes*, *E. 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.

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 UK. The upper-bound estimate of intake 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 UK 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 USA Environmental Protection Agency 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 (Environmental Protection Agency, 1998). 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.

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.

6. 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. On the basis of 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.

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