Safety Assessment of Etidronic Acid and Salts of Etidronic Acid as Used in Cosmetics

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ABSTRACT

The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) assessed the safety of Etidronic Acid, a crystalline diphosphonate, and its salts (Disodium Etidronate, Tetrapotassium Etidronate, and Tetrasodium Etidronate) as used in cosmetics. These ingredients are reported to function in cosmetics as chelating agents. The Panel noted gaps in the available safety data for the ingredients in this safety assessment. The available data on some of the ingredients are sufficient, however, especially in combination with data on sodium etidronate, trisodium etidronate, and etidronate (generic term for the acid or any simple salt), and can be used to support the safety of the entire group. The CIR Expert Panel concluded that Etidronic Acid, Disodium Etidronate, Tetrapotassium Etidronate, and Tetrasodium Etidronate are safe in cosmetics in the present practices of use and concentration described in this safety assessment.

INTRODUCTION

This is a review of the scientific literature and unpublished data available for assessing the safety of Etidronic Acid and its salts as used in cosmetics. Etidronic Acid is a crystalline diphosphonate. The other three ingredients evaluated in this report are salts of Etidronic Acid. According to the *International Cosmetic Ingredient Dictionary and Handbook* (*Dictionary*), these ingredients are reported to function in cosmetics as chelating agents (Table 1).[1] The ingredients included in this safety assessment are:

Etidronic Acid
Disodium Etidronate
Tetrapotassium Etidronate
Tetrasodium Etidronate

Pertinent data were discovered in the European Chemicals Agency (ECHA) database for Etidronic Acid and Tetrasodium Etidronate.[2,3] The ECHA website provides summaries of information submitted by industry.

Inhalation data on sodium etidronate and genotoxicity data on sodium etidronate and trisodium etidronate were included in the ECHA dossier; these data are included in this safety assessment report to provide inferences to ingredient toxicities.

In some of the literature, the name ethane-1-hydroxy 1,1-diphosphonate is used interchangeably with Etidronic Acid and its sodium salts. Several sources used the term etidronate without specifying the cation(s). When the actual ingredient tested could not be discerned, the term "etidronate" is used. Data, wherein the test material is recited as "etidronate," is likely to be useful for supporting inferences to ingredient toxicities.

CHEMISTRY

Definition and Structure

The ingredients in this report share the same structural Etidronic Acid core (Figure 1). Etidronic Acid, a crystalline diphosphonate with both detergent and chelating utilities, is a tetraprotic acid with the acidity of the first proton being rather strong, to the last being rather weak (pK_a's: pK_{a1} 1.35 ±0.08; pK_{a2} 2.87 ±0.01; pK_{a3} 7.03 ±0.01; and pK_{a4} 11.3).[4] The other three ingredients in this report are salts of Etidronic Acid (Table 1**).** Regardless of which of these four ingredients is added to a formulation, the pH of the formulation will dictate the degree of protonation *in situ*.

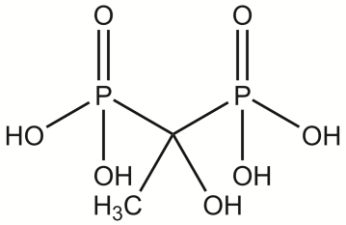


Figure 1. Etidronic Acid

Physical and Chemical Properties

In addition to the acid disassociation characteristics above, other noteworthy physical and chemical properties are provided in Table 2.

The particle size of a sample of Tetrasodium Etidronate ranged from <63 to 15,000 µm when measured using mesh sieves (Table 3).[3]

Method of Manufacture

One method of manufacture of Etidronic Acid and Disodium Etidronate starts with mixing phosphorus trichloride and acetic acid.[5] The resulting phosphorous acid anhydride mixture is heated, resulting in a condensation reaction. The product is then hydrolyzed to yield Etidronic Acid. The acid is neutralized with sodium hydroxide to create Disodium Etidronate.

Etidronic Acid can also be prepared by reaction of an acetic acid/acetic anhydride mixture with phosphorus acid.[6]

Impurities

Typical Etidronic Acid prepared for market is reported to be 95% ± 2% pure (excluding water present in commercial preparations).[7] The impurities include other organophosphates and inorganic phosphorus-containing acids (including acetic acid, hydrogen chloride, phosphonic acid, and orthophosphoric acid). Another source reported that Etidronic Acid contains up to 4% of two undisclosed phosphonic acid components.[8]

USE

Cosmetic

The safety of the cosmetic ingredients included in this safety assessment is evaluated based on data received from the U.S. Food and Drug Administration (FDA) and the cosmetic industry on the expected use of these ingredients in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in FDA's Voluntary Cosmetic Registration Program (VCRP) database. Use concentration data are submitted by Industry in response to surveys, conducted by the Personal Care Products Council (Council), of maximum reported use concentration by product category.

According to VCRP survey data received in 2017, Etidronic Acid was reported to be used in 362 formulations (12 leave-on formulations, 348 rinse-off formulations, and 2 formulations used in the bath; Table 4. Frequency of use according to duration and exposure of etidronic acid and its salts.[9,10]).[9] Tetrasodium Etidronate is reported to be used in 347 formulations (14 in leave-on formulations, 327 rinse-off formulations, and 6 formulations used in the bath). Disodium Etidronate and Tetrapotassium Etidronate are used in 11 and 2 cosmetic formulations, respectively.

The results of the concentration of use survey conducted by the Council in 2015 indicate Etidronic Acid had the highest reported maximum concentration of use; it was reported to be used at up to 0.9% in the category of other hair coloring preparations.[10] The highest maximum concentration of use reported for products resulting in leave-on dermal exposure was Etidronic Acid at 0.5% in the category of other fragrance preparations. The other ingredients are used at 0.15% or less.

All of these ingredients were reported to be used in products that come in contact with the mucous membranes (highest reported concentration of 0.5% Etidronic Acid in bath soaps and detergents); Etidronic Acid and Tetrasodium Etidronate were reported to be used in baby products (highest reported concentration of 0.026% in a body soap).

Additionally, Etidronic Acid is reported to be used in cosmetic sprays and could possibly be inhaled; it was reported to be used at up to 0.12% in the category of other fragrance preparations. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 µm, with propellant sprays yielding a greater fraction of droplets/particles <10 µm compared with pump sprays.[11,12] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and thoracic regions of the respiratory tract and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[13,14]

Etidronic Acid and its salts are currently restricted by the European Union Annex III/53 to 1.5% (as Etidronic Acid) in hair products and 0.2% (as Etidronic Acid) in soap.[15,16]

In 1987 assessment of Etidronic Acid and its salts, the European Scientific Committee on Cosmetology calculated an oral acceptable daily intake (ADI) of between 0.25 and 0.50 mg/kg (expressed as acid).[17]

Non-Cosmetic

Etidronic Acid and its salts may be used in the preparation of steam that contacts food, with the restriction that the amount of the additive does not exceed the amount required for its functional purpose, and the amount of steam in contact with the food does not exceed the amount required to produce the intended effect in or on the food.[21CFR173.310]

Etidronic Acid is exempted from the requirement of a tolerance when used in antimicrobial formulations of food-contact sanitizing solutions in accordance with good manufacturing practices (GMP), provided that (1) the substance is applied on a semi-permanent or permanent food-contact surface (other than being applied on food packaging) with adequate draining before contact with food, and (2) the end-use concentration does not exceed 14 ppm.[40CFR180.940]

Etidronic Acid may be included in sanitizing solutions that may be safely used on food-processing equipment, utensils, and other food-contact articles, when in an aqueous solution containing hydrogen peroxide, peracetic acid, acetic acid, and Etidronic Acid.[21CFR178.1010]

Etidronic Acid and its salts are used in water treatment to prevent crystalline scale deposition.[7] They are also used in detergent and cleaning applications in the paper, textiles and photographic industries and in off-shore oil well applications as complexing agents.

Etidronic Acid and its salts are used to treat bone diseases characterized by osteoclastic bone resorption including Paget's disease, hypercalcemia of malignancy, osteolytic bone metastases, and calcified uremic arteriolopathy at doses ranging from 5 to 20 mg/kg/day.[18-24]

TOXICOKINETICS

Dermal Penetration

A 5-cm^2 iontophoretic patch containing ^{14}C-Disodium Etidronate (9.8% in saline; 3 mL; 294 mg; labeled in the 1-position; specific activity 5.4 µCi/mg) was administered to the skin on the back of a pig for 76 min (including a 10-min interruption to check the pH) with a current of 4 mA.[25] The pH was adjusted to 7.4 with sodium hydroxide. Blood samples were collected hourly from a cannula for 24 h. A total of approximately 2 mg of the Disodium Etidronate penetrated the skin. At necropsy, bone samples contained 1.2 µg ^{14}C-Disodium Etidronate/g bone.

Absorption, Distribution, Metabolism, and Excretion (ADME)

Oral

Animal and human studies demonstrate that gastrointestinal absorption of Etidronic Acid and its salts is low, with the majority of the absorbed dose excreted in the feces or urine (Table 5).[2,3,8,26,27] Bone is the only tissue to exhibit deposition of radioactivity. No adverse skeletal changes were shown in subchronic and chronic studies, suggesting that there is limited toxicity. Analysis of urine from the Disodium Etidronate-treated rats and urine, feces, serum, and bone samples from the treated dogs showed that 95% to 100% of the recovered material was Disodium Etidronate.[27]

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Dermal

Dermal toxicity studies are summarized in Table 6.

In two studies, the dermal LD_{50} of Etidronic Acid in rabbits was reported to be >7940 and >10,000 mg/kg in rabbits; clinical signs at very high doses included moderate weakness, reduced appetite, and activity.[2,7,26] In two studies, the dermal LD_{50} for Tetrasodium Etidronate was >5000 mg/kg in rabbits; clinical signs at very high doses included oral and nasal discharge.[3,26]

Oral

Acute oral toxicity studies are summarized in Table 6.

In several studies, the oral LD_{50} of Etidronic Acid was reported to range from 1008 to >5000 mg/kg in rats; clinical signs included reduced appetite and activity, increasing weakness, diarrhea, tremors and collapse followed by death.[2,7] In one study, the LD_{50} in mice was reported to be 1100 mg/kg Etidronic Acid.[2,7]

In one study, the oral LD_{50} in rats of Disodium Etidronate was 1340 mg/kg; the LD_{50} for a dentifrice and a mouthwash formulation containing Disodium Etidronate was >25,000 mg/kg (equivalent to >750 mg active Etidronic Acid/kg) and 25 mg/kg (equivalent to 250 mg active Etidronic Acid/kg), respectively.[28] Over four studies, the reported oral LD_{50} of Disodium Etidronate ranged from >1000 to >5000 mg/kg in mice.[26] The oral LD_{50} of Disodium Etidronate ranged from 581 to 1140 mg/kg in rabbits at various life stages.[28] The oral median effective dose (ED_{50}) for adverse effects was 84.8 mg/kg Disodium Etidronate in dogs; varying degrees of stomach and intestinal irritation were observed at higher dosages (250 to 10,000 mg/kg), and the severity was dose-dependent.[28] Orally administered Disodium Etidronate (1% and 3%) in a dentifrice increased the emetic properties, and the ED_{50} for adverse effects of a mouthwash containing Disodium Etidronate (1%) was 5.10 mL/kg in dogs.[28]

In several additional studies, the oral LD_{50} of Tetrasodium Etidronate in rats ranged from 2850 to 5300 mg/kg (equivalent to 940 to 1290 active acid) in rats.[26] In one study, all five rats died when exposed orally to 5000 mg/kg (equivalent to 1649 mg/kg active acid).[26]

Inhalation

Acute inhalation studies of Etidronic Acid and its salts were not found in the published literature and no unpublished data were submitted.

Short-Term Toxicity Studies

Animal - Oral

Short-term oral toxicity studies are summarized in Table 7.

The no-observed-adverse-effect-level (NOAEL) for Etidronic Acid administered by gavage in rats was 30 mg/kg/day for 28 days.[2,7]

There were no differences among rats fed *ad libitum* and animals on a restricted feeding regimen during administration of etidronate (40, 200, 400, or 1200 mg/kg/day) by gavage for 4 weeks.[29] There were no differences observed between the two diets, therefore, the data were combined. Twelve rats died. Five were killed before the end of the

experiment in the 1200 mg/kg/day group because of treatment-related effects. One rat in the 400 mg/kg/day group was found dead during week 3; this was also considered treatment-related.

When cats were administered Disodium Etidronate (50 or 500 mg/kg/day), 6 of 7 in the 500 mg/kg/day group died, 5 by the end of the second week; all of the cats that died had high serum creatinine concentrations.[30] The treated cats had acute and chronic inflammation of the kidneys, involving both interstitial and tubular elements (four in the 50 mg/kg/day group and six in the 500 mg/kg/day group group).

Animal - Inhalation

Inhalation studies of Etidronic Acid and its salts were not found in the published literature and no unpublished data were submitted.

Short-term inhalation studies on sodium etidronate are included for inference purposes and are summarized in Table 8.

In repeated dose, nose-only inhalation experiments, no rats died at concentrations up to 94 mg/m^3 sodium etidronate when exposed 4 or 6 times for 4 or 5 h/day over 2 weeks.[3] Erosions were observed in the distal third of the trachea in rats treated at 0.5 mg/m^3; moderate to severe pneumonia was observed at 12 mg/m^3. All rats treated at a concentration of 94 mg/m^3 had moderate to high grade subacute or chronic laryngitis immediately after exposure that persisted through the 4-week recovery period.

Subchronic Toxicity Studies

Animal - Oral

Subchronic oral toxicity studies are summarized in **Table 7**.

The NOAEL was ≥1724 mg active acid/kg/day Etidronic Acid in female rats and 1583 mg active acid/kg/day in male rats exposed to Etidronic Acid in feed for 90 days.[2,7]

In a 91-day feeding study of Disodium Etidronate, the NOAEL was 0.2% (approximately 260 mg/kg/day) and the lowest-observed-adverse-effect-level (LOAEL) was 1% (approximately 1300 mg/kg/day) in rats.[3,28]

The oral no-observed-adverse-effect-concentration (NOAEC) of Disodium Etidronate administered in feed to rats for 90 days was 500 ppm (50 mg/kg) and the lowest-observed-adverse-effect-concentration (LOAEC) was 2000 ppm (169 mg/kg).[26]

The NOAEL of Etidronic Acid administered to Beagle dogs in feed for 90 days was ≥1746 mg active acid/kg/day in males and ≥1620 mg active acid/kg/day in females.[2,7]

Chronic Toxicity Studies

Animal - Oral

Chronic oral toxicity studies are summarized in **Table 7**.

In a 104-week feeding study in which rats were administered Disodium Etidronate (0, 19, 78, 384 mg/kg/day in males and 0, 24, 96, 493 mg/kg/day in females), the NOAEL was 24 mg/kg/day and the LOAEL was 78 mg/kg/day; clinical signs included slight and severe skin pallor.[26] When added to the drinking water of rats for 2 years, the NOAEL of Disodium Etidronate was 3.3 ppm.[26] Feed consumption and body weight increased slightly, compared with those of the control group, in rats administered radio-labeled Disodium Etidronate (134 mg/kg/day) in drinking water for 106 weeks.[3]

Five of six cats administered Disodium Etidronate at 25 mg/kg/day in feed for 6 months had both higher than normal and lower than normal serum calcium and phosphorus concentrations over the treatment period.[30] In the 1 and 10 mg/kg/day groups, only a third of the cats had hypercalcemia; although the incidence of hyperphosphatemia decreased, the incidence of hypophosphatemia increased.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY (DART) STUDIES

Oral

ETIDRONIC ACID

Pregnant Long-Evans rats (n=20) were administered Etidronic Acid (16.5, 110 or 330 mg/kg/day) by gavage on gestation days (GD) 6 through 15.[3] A half dose of the test material was administered twice per day. There were no adverse effects observed in the dams or offspring at any dose level. The maternal and teratogenic NOAELs were >330 mg/kg/day in this study. No further details were provided.

Etidronic Acid (0, 0.1%, and 0.5%) was incorporated into the feed of pregnant Charles River CD rats.[3] The stages of pregnancy at which the treatment began and ended, and whether treatment continued after parturition, were not specified. The pups and dams were killed and necropsied 56 days after weaning. One female was killed and necropsied early because of a neck tumor that appeared to be unrelated to the treatment; no other lesions were observed in the pups or the dams. The NOAEC for developmental toxicity and teratogenicity was reported to be >0.5%, the highest dose tested, in the diet.

DISODIUM ETIDRONATE

Charles River rats (n=22/sex) were administered Disodium Etidronate (0, 0.1%, and 0.5%; equivalent to 0, approximately 112, and approximately 447 mg/kg/day, respectively) in feed either (1) starting 8 weeks prior to mating

through gestation or (2) only during GD 6 to 15.[31] The F_0 females were allowed to deliver their first 2 litters; none of the $F1_a$ pups were used to produce the next generation, and 25 weanlings of each sex, from each treatment group, were selected from the $F1_b$ litters for second generation breeding stock. The third litters ($F1_c$) were used for teratogenic evaluation. All newborn pups were counted at birth, but not handled until they were 4 days old. They were then weighed, sexed, and inspected grossly. Larger litters were culled to 8 pups after the weaning weights were recorded.

For the second generation, 20 pairs of rats from each group were bred and the remaining 5 pairs were necropsied and examined histologically. The first litters from these pairs ($F2_a$) were evaluated similarly to the $F1_a$ pups (counted at birth then weighed, sexed, and inspected grossly after 4 days). The second litters ($F2_b$) were used for teratogenic evaluation. During the teratogenic examination phases, one-half of each treatment group was killed on GD 13; the other half was killed on GD 21. The dams were examined for number of resorptions, corpora lutea, and implantations. The fetuses were dried of amniotic fluid, sexed, carefully inspected for gross abnormalities and weighed. One-third of the fetuses were cleared, stained with Alizarin red, and examined for skeletal defects. The remaining two-thirds of the fetuses were examined for soft-tissue anomalies by histological methods or freehand sectioning.

The pregnancy rate of the F_0 generation was comparable to those of the control and the treatment groups. The overall conception rate was 90%. The following were observed in the first 2 litters of the F_0 female:

1. A reduction in the number of live pups born to dams of the 0.5% group exposed only during organogenesis in the $F1_a$ phase
2. An increase in the number of stillborn fetuses of $F1_b$ litters, although these dams had a higher average number of pups than the control dams

The dams of the $F1_b$ pups in the 0.1% group exposed only during organogenesis had more pups than controls and those fed the same level from before mating through gestation. Five dams died in the high-concentration groups. One death was attributed to pneumonia and another to a thyroid tumor. The cause of death could not be determined in the three other cases; in one of these three cases, the dam gave birth to 7 dead pups 2 days before dying.

There were no fetal deaths in the $F1_c$ and $F2_a$ generation. There was no increase in the number of stillborn pups in the $F1_b$ generation; there was an increase in the number of stillborn pups in the high-concentration groups of females dosed on GD 6 to 15. Other statistically significant differences were: the average number of corpora lutea decreased (11.9) compared to controls (14.2) only in the $F2_b$ generation in the continuously dosed high-concentration group; the average number of live fetuses (9.1) was reduced compared to controls (13.0) only in the high-concentration group and only in the dams dosed during organogenesis, but not in dams treated from before mating through gestation.

The authors concluded that there was no impairment of reproductive function observed in male or female rats at the concentrations and conditions tested. It was also concluded that Disodium Etidronate was not teratogenic in rats at the concentrations tested.[31]

In a 104-week feeding study of Disodium Etidronate (0, 500, 2000, 10,000 ppm; equivalent to 0, 19, 78, 384 mg/kg/day, respectively, in males and 0, 24, 96, 493 mg/kg/day, respectively, in females) in Sprague-Dawley CFY rats (n=40, 10 in satellite group), there were no histological or weight differences observed in the ovaries, uterus, or mammary gland of treated compared with untreated female rats. [26] (See Chronic Toxicity Studies [**Table 7**] for further details.)

In a range-finding study for the following experiment, female New Zealand White rabbits (n=25) were administered Disodium Etidronate (0, 100 or 500 mg/kg/day; 2 mL in water) by gavage on GD 2 to 16.[31] The two control groups were either not treated or treated with water. Does in the 500 mg/kg/day group died after day 4 or 5 of dosing; this dosage was adjusted to 250 mg/kg/day. The pregnant does were killed on GD 29 and examined for resorptions, corpora lutea, and implantations. The fetuses were dried of amniotic fluid, sexed, carefully inspected for gross abnormalities, and weighed. One-third of the fetuses were cleared, stained with Alizarin red stain, and examined for skeletal defects. The remaining two-thirds of the fetuses were examined for soft-tissue anomalies, either by histological methods or by freehand sectioning.

The untreated does were successfully inseminated (81%); 100% of the water-dosed controls, and 68% of the 100 mg group were inseminated successfully. The 4 females in the high-dose group (3 doses at 500 mg/kg/day then dose rate reduced to 250 mg/kg/day) survived to term. These does and their offspring were not included in the statistical analyses because of the treatment anomaly. There were no differences in the number of corpora lutea, implantations, resorptions, or live fetuses; there were no differences in fetal body weights. The number of live fetuses was reduced in the 100 mg/kg/day group. Of 304 control fetuses examined, seven exhibited abnormalities, including heart, eye, and urogenital defects. Six of these were in the water-dosed control group. In the 100 mg/kg/day group, two of the 99 fetuses were defective; but no defects were observed in the 18 fetuses from does in the higher dose group. Of all treated fetuses, 30% to 40% had supernumerary ribs, most of which were bilateral. Variations in the sternebrae were quite common, with atrophy of the fifth sternebrae occurring with the greatest frequency. Histopathologic examination of the does showed renal tubular degeneration in all those administered 500 and 250 mg/kg/day and in 20% of the does in the 100 mg/kg/day group. Because 50% of the control animals were similarly affected, this condition was not attributed to the Disodium Etidronate.[31]

Female New Zealand White rabbits (n=20) were administered Disodium Etidronate (0, 25, 50 or 100 mg/kg/day) in feed on GD 2 to 16.[31] To determine whether the ingestion of Disodium Etidronate in the feed might cause different effects, compared to exposure by gavage, another group of rabbits (n not specified) was administered 100 mg/kg/day by stomach tube, as was done in the preliminary study. Control groups, untreated and water-treated, were included. The does and fetuses were examined as in the preliminary study.

There were no differences in body weight gains or feed consumption attributable to treatment among groups. The group treated by gavage with 100 mg/kg/day consumed the least amount of feed and gained the least amount of weight during gestation. The overall conception rate for the 120 does used in this study was 92.5% and ranged from 85% in the non-treated control group to 100% in the water-treated controls. In comparison, the conception rates of the treated groups were 90% and 95%. Therefore, the test material had no effect on conception or on nidation. As in the pre-study, there were no differences in the numbers of corpora lutea, resorptions, or live fetuses. One doe, in the 100 mg/kg/day gavage group, aborted at 23 days and her fetuses were dead; this was attributed to severe respiratory disease. The fetuses of dams in the 100 mg/kg/day gavage group were smaller than those of untreated control dams, but the numbers of fetuses per litter was greater. Because the body weights of these fetuses did not appear to be substantially different from the body weights of the fetuses of the water-dosed control fetuses, the reduced weight was considered to be due to normal variation or to the stress of intubation. Of the 868 rabbit fetuses examined in the main study, 17 (<2%) were defective and the treated groups were not different from the controls; spina bifida and folded retina were the most frequent defects. The NOAELs for maternal toxicity, teratogenicity, and fetal toxicity were 100 mg/kg/day.[31]

GENOTOXICITY

In Vitro

In vitro genotoxicity studies are summarized in Table 9.

Etidronic Acid was not mutagenic to *Salmonella typhimurium* in an Ames assay at up to 10 µL/plate.[2,7] The results of a mammalian cell gene mutation test of Etidronic Acid (0.125 to 0.8 µL/mL) using mouse lymphoma cells were inconclusive.[2,7]

In a study included for inference purposes, sodium etidronate was not genotoxic to mouse lymphoma cells at up to 21,000 µg/mL with and without metabolic activation when exposed for 4 h, but was cytotoxic after a 24-h exposure to 3450 µg/mL.[3] In an Ames assay, trisodium etidronate was not genotoxic to *S. typhimurium* up to 2700 µg/plate with or without metabolic activation.

In Vivo

In vivo genotoxicity studies are summarized in Table 9.

Disodium Etidronate was not genotoxic in a mouse micronucleus assay when administered intraperitoneally at up to 150 mg/kg.[3,26]

Orally administered sodium etidronate, included here for potential inference purposes, was not genotoxic in a 5-day rodent dominant lethal test at up to 1000 mg/kg/day.

CARCINOGENICITY

Oral

DISODIUM ETIDRONATE

Sprague-Dawley CFY rats (n=40, 10 in satellite group), were administered Disodium Etidronate (0, 500, 2000, and 10,000 ppm; equivalent to 0, 19, 78, and 384 mg/kg/day in males, respectively, and 24, 96, and 493 mg/kg/day in females, respectively) in feed for 104 weeks.[3,26] Rats in the satellite groups were killed and necropsied at 26 and 104 weeks. All rats that died (scheduled and unscheduled) were necropsied. There were no treatment related neoplastic lesions, and those observed were considered to be normal for this strain and age.

DERMAL IRRITATION AND SENSITIZATION

Irritation

Animal

Dermal irritation studies are summarized in Table 10.

Etidronic Acid was not irritating to moderately irritating to the skin of rabbits at up to 100%.[2,7] Disodium Etidronate was not irritating or was slightly irritating to rabbits at up to 100%.[26] Tetrasodium Etidronate was not dermally irritating to rabbits at up to 33%.[3,26]

Sensitization

Animal

DISODIUM ETIDRONATE

In a guinea pig maximization assay, Disodium Etidronate (5% at induction and 25% at challenge) was administered to female Pirbright-Hartley guinea pigs (n=20).[2,26] The first administration during the induction phase was an intracutaneous injection of the test substance with Freund's Complete Adjuvant and petroleum jelly. The second administration was an epicutaneous application of the test substance in water and petroleum jelly under occlusion. The challenge was the application of the test substance in water and petroleum jelly under a semi-occlusive patch. At 24 h after the challenge, 8 test and 6 control guinea pigs showed grade 1 erythema (slight redness). At 48 h, 5 test and 2 control guinea pigs showed grade 1

erythema. At 72 h, there were no signs observed in either group. It was concluded that Disodium Etidronate was not sensitizing at 5%.

OCULAR IRRITATION STUDIES

Animal

Ocular irritation studies are summarized in Table 11.

Etidronic Acid was corrosive to the eyes of rabbits at 100%, irritating at 10% and not irritating at 1%.[2,7] Disodium Etidronate was not irritating to the eyes of rabbits at up to 20%. A dentifrice containing 10% Disodium Etidronate and a mouthwash containing 1% Disodium Etidronate were moderately irritating to the eyes of rabbits.[26,28] Tetrasodium Etidronate was a mild-to-moderate irritant to the eyes of rabbits at 23% to 30%.[26]

MUCOUS MEMBRANE IRRITATION

Dental cotton rolls with approximately 1 mL of a dentifrice or a mouthwash with and without Disodium Etidronate (3% in the dentifrice and 1% in the mouthwash) were placed on gingiva of Beagle dogs (n=3); the dog's mouths were held closed for 20 to 30 sec to ensure good contact.[28] Two treatment schedules were used: (1) the products were applied 5 times daily for 4 days, or (2) the same treatment was used but the treated areas were rinsed with 25-30 mL of tap water within 5 sec after cessation of treatment after each exposure. The treatments were continued for up to 20 days. The gingiva of each dog was examined before each treatment for signs of irritation or epithelial sloughing. Once damage to the gingiva was observed, treatment was stopped. The results for all of the treatment groups were similar to controls. The dentifrice, with and without Disodium Etidronate, caused visible irritation on day 4 without rinsing and not at all by day 20 with rinsing. Without rinsing, the mouthwash, with and without Disodium Etidronate, caused visible irritation on day 20. With rinsing, the mouthwash, with and without Disodium Etidronate, caused no damage by day 20.

CLINICAL AND RETROSPECTIVE STUDIES

Adverse Effects

In human subjects, adverse effects reported for oral medicinal exposure to etidronate (400-500 mg/day; ranging from 1-3 years) included blurred vision, ocular inflammation, gritty irritation, tearing, photophobia, orbital tenderness, and erythema; etidronate has also been linked to conjunctivitis diplopia.[32,33]

The human chronic oral studies, including adverse effects, of Etidronic Acid, Disodium Etidronate, and Etidronate are summarized in Table 12.

In humans, the reported adverse effects in clinical trials of orally administered Etidronic Acid, Disodium Etidronate, and Etidronate (usually 400 mg/day) included influenza-like symptoms, joint and bone pains, bronchitis, back pain, heartburn, constipation, cystitis, abdominal cramps, dizziness, and diarrhea.[19,20,22,34-39] The adverse events were mostly mild and transient. The subjects were administered the test substance daily for approximately 14 days (followed by calcium supplementation or no treatment for approximately 76 days) for multiple cycles.

Treatment with bisphosphonates, including etidronate, has been associated with osteonecrosis of the jaw (ONJ).[40] Analysis of ONJ cases and the associated drugs in the FDA's adverse event reporting system (FAERS) showed that these cases were associated with intravenous administration of these drugs. The odds ratio for etidronate was 12.3 (confidence interval 13.0-14.7).

Case Reports

A 26-year-old man with no significant medical history presented to the emergency room 1 h after accidental ingestion of approximately 200 mL (120 mg) of Etidronic Acid in a organophosphoric acid corrosion inhibitor product.[41] It was not specified if he consumed liquid or powder. The liquid form was described as clear and colorless with a slight odor, and contains 58% to 62% of the active chemical substance. The solid form was described as a white crystal powder that contains 95% of the active chemical substance. He had normal vital signs, physical examination, and biochemical measurements. However, 24 h later, he developed nausea with decreased urine production. His blood urea nitrogen, creatinine and uric acid levels increased to 36 mg/dL, 3.87 mg/dL, and 8.4 mg/dL, respectively. His serum calcium and phosphorus levels decreased to 7.4 mg/dL and 1.4 mg/dL, respectively. He had proteinuria, glucosuria, leukocyturia, and high phosphorus excretion in the urine. An ultrasound examination showed that the kidneys were slightly enlarged and edematous. On the third day of hospitalization, his creatinine level increased to 8.81 mg/dL and metabolic acidosis developed. He was prescribed hemodialysis therapy. Renal functions resolved without complications in 30 days.

A 12-year follow-up was reported of a 30-year-old woman who received several years of i.v. etidronate (400 mg/day for 14 days every 3 months) for pregnancy-associated spinal osteoporosis starting after the birth of her first child.[42] She received cyclic etidronate treatment for 1.5 years until her second pregnancy. After her second child was born, the treatment was resumed for approximately 2 years, discontinuing treatment 3 months prior to her third pregnancy. No neonatal complications, skeletal deformities, or hypocalcemia were observed in the woman's second and third children. However, low bone mineral density (BMD) at the lumbar spine was observed by dual-energy x-ray absorptiometry scan in the third child, who was female, at 6.8 years of age. The authors speculated that genetic predisposition may have contributed to the low

BMD value in the third child, and that the role of long-term etidronate treatment prior to conception could not be determined by this case report.

EPIDEMIOLOGICAL STUDIES

Carcinogenicity

In an open cohort study, subjects (n=22,609) who were being treated orally with etidronate (dose range was not specified) for 1 to 2 years were analyzed for the incidence of gastric and esophageal cancers. There were no increases in the incidence of gastric and esophageal cancers.[43] The rate of gastric and esophageal cancer was 48 cases per 100,000 patient years, which was lower than the background rate.

In retrospective study analyses, the use of bisphosphonates (e.g., alendronate, etidronate, and risedronate) to treat osteoporosis has been associated with the increase in cancers of the esophagus, stomach, and colorectum in humans.[44,45] However, there are other analyses indicating that there is no relationship or that there is a protective effect of bisphosphonates against esophageal or colorectal cancer.[46-51] These analyses tend to combine data from all bisphosphonates together, so that data on specific bisphosphonates, such as etidronate, are difficult to discern.

Anti-Carcinogenicity

In a retrospective study examining the relationship between the use of bisphosphonates and breast cancer, female subjects over 40 years of age with osteoporosis (n=34,103) that were treated with etidronate (doses not specified) or etidronate plus calcium were compared to the general population of women (n=261,322) over 40 years of age who were not taking these drugs over a 10-year period.[52] There was a reduced risk of breast cancer in patients treated with bisphosphonates for osteoporosis, in general, and a reduced risk associated with etidronate treatment, in particular, but there was no discernable dose-response relationship. However, most of the effect might be attributed to a relatively low endogenous cumulative exposure of estrogens, which both increase the risk of osteoporosis and decrease the risk of breast cancer. The study does not support a direct antitumor effect of bisphosphonates for breast cancer.

SUMMARY

This is a review of the available scientific literature and unpublished data relevant to assessing the safety of Etidronic Acid and its salts (Disodium Etidronate, Tetrapotassium Etidronate, and Tetrasodium Etidronate) as used in cosmetics. Etidronic Acid is a crystalline diphosphonate. These ingredients are reported to function in cosmetics as chelating agents. Studies on sodium etidronate are used for inference purposes for inhalation toxicity; studies on sodium etidronate and trisodium etidronate are used for inference purposes for genotoxicity.

The particle size of a sample of Tetrasodium Etidronate ranged from <63 to 15,000 μm.

Etidronic Acid is reported to be 95% ± 2% pure; impurities include other organophosphates and inorganic phosphorus-containing acids.

According to VCRP data received in 2017, Etidronic Acid was reported to be used in 362 formulations (12 leave-on formulations, 348 rinse-off formulations, and 2 formulations used in the bath). Tetrasodium Etidronate is reported to be used in 347 formulations (14 in leave-on formulations, 327 rinse-off formulations, and 6 formulations used in the bath). Disodium Etidronate and Tetrapotassium Etidronate are used in 11 and 2 cosmetic formulations, respectively. Based on a concentration of use survey by the Council in 2015, Etidronic Acid had the highest reported maximum concentration of use; it is used at up to 0.9% in the category of other hair coloring preparations. The highest maximum concentration of use reported for products resulting in leave-on dermal exposure was Etidronic Acid at 0.5% in the category of other fragrance preparations. The remaining ingredients are used at 0.15% or less.

There was dermal penetration of ^{14}C-Disodium Etidronate through pig skin using an iontophoretic patch. A total of approximately 2 mg of 294 mg Disodium Etidronate penetrated the skin. At necropsy, bone samples contained 1.2 μg ^{14}C-Disodium Etidronate/g bone. Animal and human studies demonstrate that gastrointestinal absorption of Etidronic Acid and its salts is low, with the majority of the absorbed dose excreted in the feces.

The dermal LD_{50} of Etidronic Acid in rabbits was reported to range from >7940 to 10,000 mg/kg in rabbits; clinical signs included moderate weakness, reduced appetite, and activity. The dermal LD_{50} for Tetrasodium Etidronate was >5000 mg/kg in rabbits; clinical signs included oral and nasal discharge.

The oral LD_{50} of Etidronic Acid was reported to range from 1008 to 5000 mg/kg in rats; clinical signs included reduced appetite and activity, increasing weakness, diarrhea, tremors and collapse followed by death. The LD_{50} in mice was reported to be 1100 mg/kg Etidronic Acid.

The oral LD_{50} in rats of Disodium Etidronate was 1340 mg/kg; the LD_{50} for a dentifrice and a mouthwash formulation containing Disodium Etidronate was 25 g/kg (equivalent to 750 mg active Etidronic Acid/kg) and 25 mg/kg (equivalent to >250 mg active Etidronic Acid/kg), respectively. The oral LD_{50} of Disodium Etidronate ranged >1000 to >5000 mg/kg in mice. The oral LD_{50} of Disodium Etidronate ranged 581 to 1140 mg/kg in rabbits. The oral ED_{50} was 84.8 mg/kg Disodium Etidronate in dogs; at higher doses (0.25 to 10 g/kg) varying degrees of stomach and intestinal irritation were observed, and severity was dose-dependent. Orally administered Disodium Etidronate (1% and 3%) in a dentifrice increased the emetic properties of the dentifrice, and the ED_{50} of a mouthwash containing Disodium Etidronate (1%) was 5.10 mL/kg in dogs for vomiting.

The oral LD_{50} of Tetrasodium Etidronate in rats ranged from 2850 to 5300 mg/kg (equivalent to 940 to 1290 active acid) in rats. In one study, all five rats died when orally administered 5000 mg/kg (equivalent to 1649 mg/kg active acid).

The NOAEL for Etidronic Acid administered by gavage in rats for 28 days was 30 mg/kg/day; the NOAEL was 1724 mg active acid/kg/day in female rats and 1583 mg active acid/kg/day in male rats when administered in feed for 90 days. The NOAEL of Etidronic Acid administered in feed for 90 days for Beagle dogs was 1746 mg/kg/day active acid/kg for males and 1620 mg/kg/day active acid/kg for females.

The oral NOAEC of Disodium Etidronate administered in feed to rats for 90 days was 500 ppm (50 mg/kg/day) and the LOAEC was 2000 ppm (169 mg/kg/day).

There were no differences between animals fed *ad libitum* and animals on a restricted feeding regimen during administration of etidronate (40, 200, 400, or 1200 mg/kg/day) by gavage for 4 weeks.

In a 91-day feeding study, the NOAEL was 0.2% (approximately 260 mg/kg/day) and the LOAEL was 1% (approximately 1300 mg/kg/day) for rats. In a 104-week feeding study of rats, the NOAEL for Disodium Etidronate was 24 mg/kg/day and the LOAEL was 78 mg/kg/day (clinical signs included slight and severe skin pallor). When added to the drinking water of rats for 2 years, the NOAEC was 3.3 ppm Disodium Etidronate. Feed consumption and body weight increased slightly in comparison with the control group in rats administered radio-labeled Disodium Etidronate (0.84 mg/kg/day) in drinking water for 106 weeks.

When cats were administered Disodium Etidronate, six of seven in the 500 mg/kg/day group died, 5 by the end of the second week. Five of six cats administered Disodium Etidronate at 25 mg/kg/day in feed for 6 months had both higher than normal and lower than normal serum calcium and phosphorus values over the treatment period.

In repeated dose, nose-only inhalation experiments, no rats died when administered up to 94 mg/m^3 sodium etidronate. Erosions were observed in the distal third of the trachea in rats treated at 0.5 mg/m^3; moderate to severe pneumonia was observed at 12 mg/m^3.

The NOAEL was 330 mg/kg/day for pregnant rats administered Etidronic Acid by gavage on GD 6 through 15. The NOAEC for developmental toxicity and teratogenicity was >0.5% Etidronic Acid in the diet of rats. At necropsy, there were no abnormalities attributable to the test substance in the reproductive organs of rats or dogs administered Etidronic Acid in the diet up to 30,000 or 10,000 ppm, respectively, for 90 days.

In a multi-generational study, Disodium Etidronate was not teratogenic to rats at up to 447 mg/kg/day administered in feed starting 8 weeks prior to mating; the NOAEL was 112 mg/kg/day for both the F0 and F1 generations. The NOAEL for effects on reproductive organs were 384 mg/kg/day Disodium Etidronate in male rats and ≥493 mg/kg/day in female rats. New Zealand White rabbits administered Disodium Etidronate up to 250 mg/kg/day by gavage on GD 2 through 16 did not exhibit increases in the instances of abnormalities in the offspring; 21 of 25 does administered 500 mg/kg/day died on day 4 or 5 of dosing. The NOAEL for maternal toxicity, teratogenicity, and fetal toxicity were 100 mg/kg/day in rabbits administered Disodium Etidronate in feed on GD 2 through 16.

Etidronic Acid was not mutagenic to *S. typhimurium* in an Ames assay at up to 10 µL/plate. The results of a mammalian cell gene mutation test of Etidronic Acid (0.125 to 0.8 µL/mL) using mouse lymphoma cells were inconclusive. Sodium etidronate was not genotoxic to mouse lymphoma cells at up to 21,000 µg/mL, with and without metabolic activation when exposed for 4 h, but was cytotoxic at 3450.0 µg/mL when exposed 24 h. In an Ames assay, trisodium etidronate was not genotoxic to *S. typhimurium* at up to 2700 µg/plate with and without metabolic activation.

Disodium Etidronate was not genotoxic in a mouse micronucleus assay at up to 150 mg/kg when administered intraperitoneally. Orally administered sodium etidronate was not genotoxic in a rodent dominant lethal test at up to 1000 mg/kg/day for 5 days.

A carcinogenic effect was not observed when 384 and 493 mg/kg/day Disodium Etidronate was administered in feed for 104 weeks of male and female rats, respectively.

Etidronic Acid was not dermally irritating to moderately irritating to rabbits at 100%. Disodium Etidronate was not irritating to rabbits at 20% and slightly irritating at 100%. Tetrasodium Etidronate was not irritating to rabbit skin at 30% - 33%.

Disodium Etidronate was not sensitizing in a guinea pig maximization assay at 5%.

Etidronic Acid was corrosive to the eyes of rabbits at 100%, irritating at 10% and not irritating at 1%. Disodium Etidronate was not irritating to the eyes of rabbits at up to 20%; a dentifrice containing 10% Disodium Etidronate and a mouthwash containing 1% Disodium Etidronate were moderately irritating to the eyes of rabbits. Tetrasodium Etidronate was a mild to moderate irritant to the eyes of rabbits at 23% to 30%.

Medicinal exposure to etidronate (ranging from 1-3 years) has been reported to cause blurred vision, probably causes conjunctivitis, and possibly causes diplopia in human subjects. In clinical trials of Etidronic Acid, Disodium Etidronate, and etidronate (usually 400 mg/day), where the subjects were orally administered the test substance daily for approximately 14 days followed by daily calcium or no treatment for approximately 76 days for multiple cycles, the adverse events included influenza-like symptoms, joint and bone pains, bronchitis, back pain, heartburn, constipation, cystitis, abdominal cramps, dizziness, and diarrhea. The adverse events were mostly mild and transient.

There is no correlation between the treatment of humans for osteoporosis with bisphosphonates, including etidronate, and breast, esophagus, stomach and colorectal cancers.

DISCUSSION

The Panel examined data for Etidronic Acid and its salts (e.g., Disodium Etidronate, Tetrapotassium Etidronate, and Tetrasodium Etidronate). The Expert Panel noted gaps in the available safety data for Etidronic Acid and some of its salts in this safety assessment. Chemical and biological effects of etidronate salts are driven by the etidronate molecule, which is readily formed by dissociation of the cations in aqueous solution. Therefore, the Panel found the data on the etidronate salts that were not cosmetic ingredients to be appropriate for inference on the safety of the cosmetic ingredients named in this safety assessment.

In some studies, no irritation or mild irritation was observed at concentrations well above the levels of expected use of these salts. Mucous membrane irritation was only observed with frequent contact, without rinsing, of a dentifrice or a mouthwash, which is not the intended mode of use. A single sensitization study of Disodium Etidronate, conducted at well above expected use levels, showed no signs of sensitization. In addition, there is significant data on the long-term oral clinical use of these ingredients where the favorable overall safety profile is consistent with therapeutic use at doses that far exceed those in cosmetic ingredient use.

These ingredients are not expected to absorb UV light, thus, the Panel was not concerned with the lack of photosensitization or phototoxicity data.

The Panel noted that the methodology used in the dermal penetration study, which included the use of an iontophoretic patch, promotes dermal penetration. Because the study results exaggerated the dermal penetration of Disodium Etidronate and the tested dose was not representative of exposure to this ingredient in cosmetic use, the amount of dermal penetration that is expected is not of concern.

The Panel discussed the issue of incidental inhalation exposure from fragrance preparations. There are limited data on sodium etidronate that suggest little potential for respiratory effects at relevant concentrations. These ingredients are reportedly used at concentrations up to 0.12% in cosmetic products that may be aerosolized. The Panel noted that 95% to 99% of droplets/particles would not be respirable to any appreciable amount. The potential for inhalation toxicity is not limited to respirable droplets/particles deposited in the lungs. In principle, inhaled droplets/particles deposited in the nasopharyngeal and thoracic regions of the respiratory tract may cause toxic effects depending on their chemical and other properties. Coupled with the small actual exposure in the breathing zone and the concentrations at which the ingredients are used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at http://www.cir-safety.org/cir-findings.

CONCLUSION

The CIR Expert Panel concluded that Etidronic Acid, Disodium Etidronate, Tetrapotassium Etidronate, and Tetrasodium Etidronate are safe in cosmetics in the present practices of use and concentration described in this safety assessment.

TABLES

Table 1. Definitions, structures, and functions of etidronic acid its salts.[1, CIR Staff]

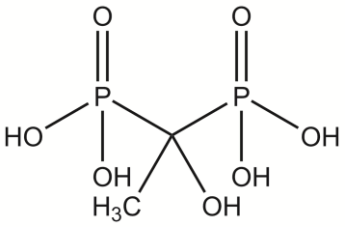
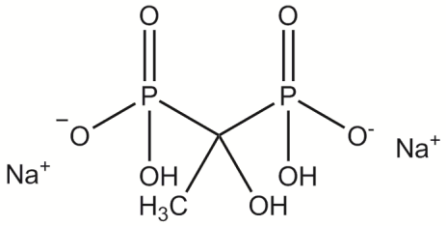
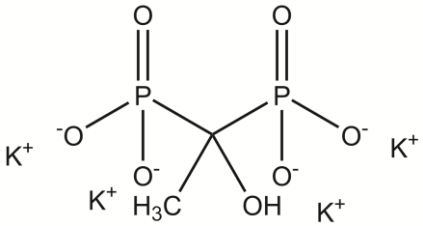
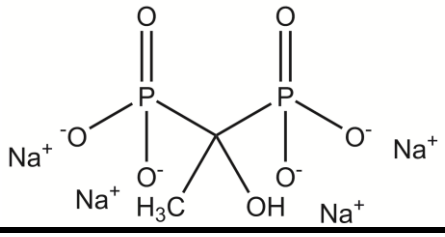
Ingredient CAS No.	Definition & Structure	Function
Etidronic Acid 2809-21-4	Etidronic Acid is the organic diphosphonic acid that conforms to the formula: 	Chelating agent
Disodium Etidronate 7414-83-7	Disodium Etidronate is the organic compound that conforms to the formula: 	Chelating agent
Tetrapotassium Etidronate 14860-53-8	Tetrapotassium Etidronate is the diphosphonic acid derivative that conforms to the formula: 	Chelating agent
Tetrasodium Etidronate 3794-83-0	Tetrasodium Etidronate is the diphosphonic acid derivative that conforms to the formula: 	Chelating agent

Table 2. Chemical and physical properties of Etidronic Acid and Its Salts.

Property	Value	Reference
Etidronic Acid		
Physical Form	Solid[a]	[7]
Formula Weight g/mol	206.03	[4]
Density/Specific Gravity kg/m^3 @ 20^{o}C	1450-1490	[2]
Vapor pressure mmHg @ 25^{o}C	1.24x10^{-10}	[53]
Melting Point ^{o}C	198-199	[7]
Boiling Point ^{o}C	457 est.	[7]
Water Solubility g/L @ 20 ^{o}C	690	[2]
@ 25 ^{o}C	680	[2]
log P_{ow}	-3.50	[2]
Disassociation constants (pKa) @ ^{o}C		
pK_{a1}	1.35 ±0.08	[4]
pK_{a2}	2.87 ±0.01	
pK_{a3}	7.03 ±0.0	
pK_{a4}	11.3	
Disodium Etidronate		
Physical Form	Solid	[53]
Formula Weight g/mol	249.99	[4]
Density kg/m^3 @ 20˚C	100-200	[26]
Vapor pressure mmHg @ 25^{o}C	2.06x10^{-11} est.	[53]
Melting Point ^{o}C	480est.	[53]
Boiling Point ^{o}C	90 est.	[53]
Water Solubility g/L @ 25^{o}C	1x10^{+6} est.	[53]
log K_{ow}	-4.74 est.	[53]
Tetrapotassium Etidronate		
Physical Form	Dry powder	[54]
Formula Weight g/mol	358.39	[54]
Vapor pressure mmHg @ 25^{o}C	1.78x10^{-10} est.	[53]
Melting Point ^{o}C	480.0 est.	[53]
Boiling Point ^{o}C	90.3 est.	[53]
Water Solubility g/L @ 25^{o}C	1x10^{+6}	[53]
log K_{ow}	-8.12 est.	[53]
Tetrasodium Etidronate		
Physical Form	Powder	[3]
Color	White	[3]
Odor	Odorless	[3]
Formula Weight g/mol	293.95 cal.	[55]
Density/Specific Gravity @ 20 ^{o}C	2.074	[3]
Vapor pressure mmHg @ 20 ^{o}C	1.575	[3]
Boiling Point ^{o}C	>103	[3]
Water Solubility g/L @ 20^{o}C	690	[7]
@ 25 ^{o}C	680	[7]
@ 20^{o}C & pH 10.4	774	[3]
log P_{ow} @ 23˚C	0.001	[3]

[a] Manufactured as aqueous solution
cal.=calculated. est.=estimated.

Table 3. Particle size distribution of a sample of Tetrasodium Etidronate using mesh sieves.[3]

Size (µm)	<63	63-125	125-250	250-500	500-1000	1000-1400	1400-15,000
Distribution (%)	1.3	2.4	5.8	13.8	22.2	17.9	36.6

Table 4. Frequency of use according to duration and exposure of etidronic acid and its salts.[9,10]

Use type	Uses	Maximum Concentration (%)	Uses	Maximum Concentration (%)	Uses	Maximum Concentration (%)	Uses	Maximum Concentration (%)
	Etidronic Acid		Disodium Etidronate		Tetrapotassium Etidronate		Tetrasodium Etidronate	
Total/range	**362**	**0.0014-0.9**	**11**	**0.04-0.097**	**2**	**0.097**	**347**	**0.009-0.15**
Duration of use[a]								
Leave-on	12	0.0014-0.12	1	NR	NR	NR	14	0.02-0.06
Rinse-off	348	0.008-0.9	10	0.04-0.097	2	0.097	327	0.009-0.15
Diluted for (bath) use	2	NR	NR	NR	NR	NR	6	NR
Exposure type								
Eye area	NR	NR	NR	NR	NR	NR	NR	NR
Incidental ingestion	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-sprays	1;10[b]	0.082-0.12; 0.0014-0.1[c]	1[b]	NR	NR	NR	5[c]; 1[b]	0.02[c]
Incidental inhalation-powders	10[b]	0.05[d]	1[b]	NR	NR	NR	1[b]	NR
Dermal contact	108	0.011-0.5	11	0.04-0.097	2	0.097	340	0.009-0.15
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair-noncoloring	18	0.0014-0.66	NR	NR	NR	NR	2	0.02-0.14
Hair-coloring	216	0.01-0.9	NR	NR	NR	NR	5	NR
Nail	20	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	90	0.033-0.5	8	0.04-0.097	2	0.097	291	0.02-0.15
Baby	NR	0.011-0.026	NR	NR	NR	NR	3	NR

NR = Not Reported; Totals = Rinse-off + Leave-on + Diluted for Use Product Uses.

[a] Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

[b] Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation.

[c] It is possible these products may be sprays, but it is not specified whether the reported uses are sprays.

[d] It is possible these products may be powders, but it is not specified whether the reported uses are powders.

Table 5. Oral ADME studies of Etidronic Acid and Disodium Etidronate.

Animal (n)	Dosing and procedure	Results	Reference
Etidronic Acid			
Rats (n and strain not specified)	0.5, 5.0, 10, 50 and 200 mg/kg aqueous ^{14}C-Etidronic Acid administered by gavage with and without fasting. Intestinal absorption was determined from collected feces and urine, bone samples, and residual body homogenate over 72 h.	Absorption in nonfasted rats ranged from 1.28% to 2.62%. Fasted animals absorbed about one-third more than did fed animals in all dose groups. 2.7% of dose was found in bones of fed rats and 8.8% in fasted rats. Most of test substance was excreted in urine.	2
Rats (n and strain not specified)	0.3 mg/kg ^{14}C-Etidronic Acid administered to fasted rats preceded or followed by administration of milk (dose not specified). Adsorption to bone and elimination in urine and feces were determined.	Adsorption to bone was reduced by a factor of 20 when milk was administered before Etidronic Acid, compared to milk administration after dosing. 0.1%-0.2% (0.3-0.6 ppb/kg) of Etidronic Acid was absorbed when administered to non-fasting rats.	2
Human (n=5,3)	Single oral dose of ^{14}C-Etidronic Acid (0.3 mg/kg) before and after breakfast. Absorption was determined by measuring radioactivity in urine.	Activity excreted in urine was 0.082% and 0.532% of applied dose when administered before and after breakfast, respectively.	2
Disodium Etidronate			
Male Wistar rats (n=150, 75 controls)	Radio-labeled Disodium Etidronate (1-^{14}C-(Disodium Etidronate) (tetrahydrate)) administered in drinking water over 2 years, 0.184 mg/kg/day with a total intake of 134 mg/kg. 4 test rats and 2 control rats were killed and necropsied every 4 weeks; sampling for presence of radioactivity was conducted on blood, organs, and tibia bones. After test period, remaining rats had a recovery period of 33 weeks then were also killed and necropsied.	Disodium Etidronate in tissues was barely above detection level; most detectable Disodium Etidronate was in intestines (40.70% of the retained radioactivity) and bones (54.30%). At 4 weeks, proportion of Disodium Etidronate in total skeleton was 0.033% of the administered radioactivity; by end of test period, proportion decreased to 0.0065%. Amount of Disodium Etidronate in skeleton decreased during recovery period. It was concluded that only a small proportion of Disodium Etidronate administered in drinking water was absorbed in intestinal tract and absorbed to bones.	3

Table 5. Oral ADME studies of Etidronic Acid and Disodium Etidronate.

Animal (n)	Dosing and procedure	Results	Reference
Male Wistar rats (n=4)	^{14}C-Disodium Etidronate (50 mg/kg; 1.520 µCi/mg; position of radio-labeling not specified) was administered via gavage. Feces and urine were collected at 6, 24, 48 and 72 h. Rats were then killed and gastrointestinal tract was dissected. Radioactivity was measured in feces, urine, gastrointestinal tract, and remaining carcass.	Disodium Etidronate was mainly excreted in feces (> 90% of the recovered radioactivity or > 80% of applied dose at 72 h). The elimination half-life was 6.2 h. Intestinal absorption was 5.98 % of applied dose. The half-life of renal elimination of Disodium Etidronate was 9 h. Approximately 90% of total eliminated radioactivity was found in urine in first 6 h. At 72 h, organs of gastrointestinal tract contained a total of 0.0772% of applied dose. Highest concentration was found in small intestine (0.0600%).	3,26
Sprague-Dawley rats (n=4); Weanling rats (n=3)	^{14}C-Disodium Etidronate (50 mg/kg; position of radio-labeling not specified) administered by gavage to young adult rats that had been eating a base diet or diet containing Disodium Etidronate (0.5%) for 30 days. Administered by gavage to weanling rats.	Most of recovered test material was in fecal matter (92.7% and 84% without and with the test substance in the diet, respectively). Recovered test material totaled 2.8% and 3.6% (from the urine, kidney, liver, carcass, and CO_2) without and with the test substance in the diet, respectively. Weanling rats had most of recovered test material in their fecal matter (78.1%). When young adult rats were administered ^{14}C-Disodium Etidronate in various amounts, most of test material was recovered in feces (89.1% to >100%) at 0.1 to 10 mg. At 200 mg, only 59.4% was recovered in feces and total amount recovered was 75.5%, indicating that most of test substance was not absorbed. Increase in absorption may be attributed to damage to gastrointestinal tract induced by test substance.	27
Male New Zealand White rabbits (n=3)	^{14}C-Disodium Etidronate (50 mg/kg; position of radio-labeling not specified) administered by gavage	An average of 78% of test material was recovered in feces, 25.8% in intestinal tract, and 3.8% in urine, kidney, liver, carcass, and CO_2.	27
4-6 months old, female Beagle dogs (n=3)	^{14}C-Disodium Etidronate (50 mg/kg; position of radio-labeling not specified) administered by gavage	An average of 80.9% of test material was recovered in feces, 2.4% in intestinal tract, and 21.1% in urine, kidney, liver, carcass, and CO_2.	27
Young (4-6 months old; n=8) and adult (5-8 years old; n=4) female Beagle dogs	^{32}P-Disodium Etidronate (50 mg/kg) was administered by gavage	An average of 60.4% and 81.3% of test material was recovered in feces of young and adult dogs, 0.1% and none in intestinal tract, and 20.6% and 13.6% in urine, kidney, liver, carcass, and CO_2, respectively.	27
Rhesus monkeys (n=1, 2)	^{14}C-Disodium Etidronate (50 mg/kg; position of radio-labeling not specified) or ^{32}P-Disodium Etidronate (20 mg/kg; position of radio-labeling not specified) administered by gavage	Recovery of test materials (^{14}C- and ^{32}P-labeled) in feces was 97% and 91.5%; 0.05% and 0.3% in intestinal tract; and 0.6% and 6.2% in urine, kidney, liver, carcass, and CO_2, respectively.	27
Humans (n=6 male, 9 female)	Orally administered 3 doses of ^{14}C-Disodium Etidronate (0.1, 1.0, and 5 mg/kg; radio-labeled at C-1 position) in capsules. Time between doses was not specified. When necessary, radioactive test substance was diluted with unlabeled Disodium Etidronate so that all test subjects received same absolute dose of radioactivity.	Intestinal absorption of Disodium Etidronate was 1.47% to 2.65%; 62.7% to 83.0% of administered material was eliminated in feces. Elimination of majority of absorbed test substance in urine occurred within 1 day.	3
Humans (group 1=4, group 2=5)	Orally administered Disodium Etidronate (30 mg/kg/day; position of radio-labeling not specified) for 2 weeks. 48 h after last dose, group 1 was orally administered ^{14}C-Disodium Etidronate (5 mg/kg; 4.5 mCi/g; disodium ethane-1-hydroxy-1,1-diphosphonate-1-^{14}C) and group 2 was orally administered ^{14}C-Disodium Etidronate (30 mg/kg) as a dry powder suspended in water after an overnight fast. Blood samples were collected in both groups over 5 and 8h, respectively. In group 2, urine samples were collected over next 24 h and stool samples were collected over next 5 d.	It was estimated that intestinal absorption in group 1 was 3.35% and 7.19% in group 2; no further results were provided.	8
Female humans with osteoporosis (n=10)	Orally administered Disodium Etidronate (20 mg/kg/day) for 6 months; half of these subjects continued treatment for an additional 6 months. Intestinal absorption and urinary excretion was determined at 6 and 12 months.	Intestinal absorption of Disodium Etidronate was highly variable; mean uptake after 6 months of treatment was 10.0% (range 0.0%-17.7%) and after 12 months of treatment was 10.7% (range 1.5%-18.2%). Overall mean absorption for both study phases was 10.3% ± 5.7%.	21
Humans (n=10)	Orally administered ^{32}P-Disodium Etidronate (40 µCi in a 20 mg/kg Disodium Etidronate carrier dose; position of radio-labeling not specified).	Recovery of test materials over 6 days after exposure averaged 70%-90%; no further results were provided.	56

Table 6. Acute toxicity studies of Etidronic Acid and Its Salts.

Animal (n if specified)	Details	Results	Reference
Dermal			
Etidronic Acid			
New Zealand White rabbits, male or female (n=1/dose)	1000, 1580, 2510, 3980, 6310 and 10,000 mg/kg (equivalent to 600, 948, 1506, 2388, 3786 and 6000 mg active acid/kg, respectively) under occlusion for 24 h. 6310 and 10,000 mg/kg were administered over a 2-h period. Test substance was sprayed with water every 2 h for first 8 h to prevent drying.	No rabbits died. Clinical signs: moderate weakness and much discomfort at higher dosage levels but no paralysis developed. There was some loss of weight at 3 highest doses. LD_{50} >10000 mg/kg (estimated to be equivalent to >6000 mg active acid/kg by one reviewer, >2300 mg/kg by another)	2,7,26
New Zealand White Rabbits, (low-dose group=1 male and high-dose group=1/sex)	5010 or 7940 mg/kg for 24 h, then observed for 14 days. No further details were provided.	No rabbits died. Clinical signs: Reduced appetite and activity within 2-3 days after treatment. Necropsy: viscera appeared normal. LD_{50} >7940 mg/kg (equivalent to 4764 mg active acid/kg)	2,7
Tetrasodium Etidronate			
New Zealand White rabbits, male and female; 8 weeks old (n=5)	5000 mg/kg (31% aqueous; equivalent to 1650 active acid), covering 10% of body surface, for 24 h under occlusion. Excess test substance was wiped off. Rabbits were observed for 14 days.	1 female died on day 13 (necropsy revealed signs of intestinal disease, suggestive of mucoid enteritis, not related to test substance). 4 rabbits survived through observation period; all surviving rabbits had some occurrences of oral and nasal discharge. Most rabbits had slight weight losses at days 7 and/or 14. Other than dermal lesions, there were no abnormal findings in other rabbits. LD_{50} >5000 mg/kg.	3,26
New Zealand White rabbits (n=1/sex/group)	1000, 1580, 2510, 3980, 6310 and 10000 mg/kg mg/kg (31% aqueous), covering 10% of body surface, for 24 h under occlusion. Excess test substance was wiped off. Rabbits were observed for 14 days.	No rabbits died. Clinical signs: considerable weakness in 2 highest-dose groups, lethargy and reduced appetite for several days. No paralysis developed. LD_{50} >5000 mg/kg (equivalent to >2300 active acid).	3
Oral			
Etidronic Acid			
Mice (strain and n not specified)	60% in water.	LD_{50}=1100 mg/kg	2,7
Sprague-Dawley rat, male or female (n=5)	2000, 2510, 3160 and 3980 mg/kg (1200, 1506, 1896 and 2388 mg active acid/kg; 50% in water)	0, 1, 3, 4 deaths at 2000, 2510, 3160 and 3980 mg/kg, respectively. Survival time was 1-8 h; most deaths occurred in 1-2 h. Clinical signs: weakness within minutes of administration followed by dyspnea and collapse. Necropsy: in animals that died, inflammation of gastric mucosa and hemorrhagic lesions in lungs were observed. LD_{50}=3130 mg/kg (1878 mg active acid/kg)	2,7
Wistar rat, female (n=10)	2510, 3160, 3570 and 3980 mg/kg	0, 1, 6, 10 deaths at 2510, 3160, 3570 and 3980 mg/kg, respectively. Unspecified clinical signs occurred at all doses. LD_{50}=3500 mg/kg	2,7
Sprague-Dawley rats, male or female (n=5)	1580, 2000, 2510 and 3160 mg/kg (doses equivalent to 948, 1200, 1506 and 1896 mg active acid/kg). Observed for 14 days.	0, 1, 3, 4 deaths at 1580, 2510, 3160 and 3980 mg/kg, respectively. Clinical signs: reduced appetite and activity (1-2 days), increasing weakness, diarrhea, tremors and collapse followed by death. Necropsy: lung and liver hyperemia and acute gastrointestinal inflammation. Viscera of survivors appeared normal. LD_{50}=2400 mg/kg (1440 mg active acid/kg)	2,7
Wistar rat, male or female (n=5/sex)	1.33; 1.47; 1.62; 1.78; 1.96 and 2.15 mL/kg. Observed for 14 days.	Clinical signs: A few minutes after administration rats were apathetic. Locomotion was reduced and characterized by ataxic behavior. Approximately 60 min later, paralysis of rear legs was observed. Strong convulsions were observed before death. Necropsy: Rats that died showed intestinal hemorrhage and gastric bleeding. Livers were brighter compared to controls. Internal organs of surviving rats appeared normal. Male LD_{50}=2442 mg/kg; female LD_{50}=2268 mg/kg.	2
Wistar rat, male (n=5)	500, 1000 and 2000 mg/kg. Observed for 14 days.	Clinical signs: convulsion, lying down on abdomen and sides, piloerection (at 1000 and 2000 mg/kg), tail phenomenon (at 2000 mg/kg). At 24 h, 3 rats died in 1000 mg/kg group and 4 died in 2000 mg/kg group. LD_{50}=1008 mg/kg	2
Wistar rat, female (n=5)	500, 1000, 2000 mg/kg	Clinical signs: at 1000 mg/kg in approximately 24 h, piloerection was noted; at 2000 mg/kg at 4 h, lying down on abdomen and sides, piloerection, tail phenomenon, and convulsions were noted. In 2000 mg/kg group, 2 rats died at 24 h and 1 died at 7 d. In 1000 mg/kg group, 1 died at 14 d. LD_{50}=1703	2
Rat (not specified)	Up to 5000 mg/kg	LD_{50} >5000 mg/kg	2

Table 6. Acute toxicity studies of Etidronic Acid and Its Salts.

Animal (n if specified)	Details	Results	Reference
Disodium Etidronate			
Mouse	Not specified	LD_{50} >1000 mg/kg	26
Mouse	Not specified	LD_{50}=3370 mg/kg	26
Mouse	Not specified	LD_{50} >5000 mg/kg	26
Mouse	Not specified	LD_{50}=3300 mg/kg	26
Charles River CD rats (n=10)	814, 1140, and 1600 mg/kg in water; 25 g/kg of a dentifrice formulation (3%; equivalent to 750 mg active acid); and 25 g/kg of a mouthwash formulation (1%; equivalent to 250 mg active acid) administered by gavage after fasting. Rats were observed for 14 days.	Numbers of deaths were not reported. At necropsy: 3 of 10 surviving rats in mid- and high-dose groups had pale (light grey), granular kidneys. Histology: changes appeared to involve all parts of kidney. Low-dose group had mild tubular changes. High-dose group had up to 20% to 40% kidney tubular changes in all rats. Mucosal irritation was observed in stomachs of 4 and 10 rats examined from mid- and high-dose groups. LD_{50}=1340 mg/kg. LD_{50} for dentifrice was 25 g/kg (equivalent to 750 mg active acid/kg) and LD_{50} for mouthwash formulation was 25 mg/kg (equivalent to 250 mg active acid/kg).	28
New Zealand White rabbits (immature=2/sex; immature=2/sex; mature-virgin=4 females; pregnant=4 females; mature=4 males)	Doses and vehicle not specified. Administered by gavage after fasting. Rabbits were observed for 14 days. 4 groups of rabbits were used in each test. 2 sets of immature rabbits were used in separate runs.	Number of deaths not reported; deaths occurred on days 1-4 with most occurring within 24 h. Diarrhea was observed at 4-48 h post-treatment, severity generally in proportion to dosage. Several rabbits in all groups appeared to have mild gastric irritation. At necropsy: approximately 50% of surviving rabbits from all groups had kidney lesions resembling chronic interstitial nephritis. [Note: Because chronic interstitial nephritis is common in rabbit, it is difficult to identify test-related symptoms.] LD_{50}s at different life stages: Immature=1140 mg/kg (CI=829-1560 mg/kg) Immature=1050 mg/kg (CI=905-1210 mg/kg) Combined=1100 mg/kg (CI=961-1520 mg/kg) Mature, virgin=814 mg/kg (CI=642-1030 mg/kg Pregnant=668 mg/kg (CI=542-872 mg/kg) Mature males=581 mg/kg (CI=424-799 mg/kg) Combined=700 mg/kg (CI=561-831 mg/kg)	28
Beagle dogs (n=2/sex)	Fasted dogs were administered Disodium Etidronate alone, a mouthwash containing 1.00% Disodium Etidronate or toothpaste containing 1.00% or 3.00% Disodium Etidronate by gavage. Dogs were observed for 4 h then allowed to eat and drink. Dogs were observed for 14 days thereafter. 2 additional dogs were administered 10 g/kg toothpaste (1 and 3 g Disodium Etidronate), observed for 1 week, then killed and necropsied.	Disodium Etidronate ED_{50}=84.8 mg/kg, CL=31.8-226 mg/kg. At higher doses (0.25 to 10 g/kg) varying degrees of stomach and intestinal irritation were observed, severity was dose-dependent. Bloody vomit, diarrhea, and loss of appetite were observed in males at 0.50 g/kg and in females at 1.0 g/kg. LD_{50} for Disodium Etidronate was estimated to be 1.0 g/kg based on early deaths and necropsy of moribund dogs dosed at higher dosages (1.0-10 g/kg). Disodium Etidronate increased emetic properties of dentifrice; ED_{50} at 1% and 3% were 1.21, and 0.681 g/kg, respectively. In all cases, dogs appeared normal after cessation of vomiting. ED_{50} of mouthwash was 5.10 mL/kg. Only clinical sign was emesis. At necropsy, there were no microscopic lesions that could be attributed to treatment; all hematological values were within normal range for dogs.	28
Tetrasodium Etidronate			
Sprague-Dawley rat, male or female (n=5)	5000 mg/kg (1649.12 active acid)	All rats died within 7 h	26
Sprague-Dawley rat (n=5/sex)	2000, 2500, 3200 and 4000 mg/kg; OECD TG 401 (Acute Oral Toxicity) with no post-dose fast	1, 4, 7 and 8 rats died, respectively. Clinical signs in all groups up to 4 h post-dosing: ataxia and/or tremors, oral and nasal discharge, hypoactivity, soft stool and fecal and/or urinary staining. Most of surviving rats had some weight loss during first week after dosing; all rats gained weight between days 7 and 14. Necropsy: macroscopic abnormalities were primarily discolorations in lungs and gastrointestinal tract (red or black walls, or red or black fluid present, suggestive of an irritant effect). Most rats in 2500, 3200 and 4000 mg/kg groups had enlarged kidneys, and 1 rat in 4000 mg/kg group had unilateral renal pallor, dilated renal pelvis and red fluid surrounding kidney. LD_{50}=2850 mg/kg (equivalent to 940 mg active acid/kg).	26
Sprague-Dawley rat, male and female (n=2 or 3/sex)	3980, 5010, 6310 and 7940 mg/kg (50% aqueous)	0, 2, 5 and 5 rats in each group died, respectively. Survival time was 2-24 h with most deaths occurring within 24 h. Clinical signs: rapid weakness with collapse in 10-30 min, diarrhea, and convulsions. Some rats that collapsed were improved in 1-2 h and survived. Necropsy: macroscopic examination showed renal, liver and pulmonary hyperemia. LD_{50}=5300 mg/kg (equivalent to 1219 mg/kg active acid)	26

CL=Confidence limits; OECD TG =Organisation for Economic Co-operation and Development Test Guideline

Table 7. Short-term, subchronic, and chronic oral toxicity animal studies of Etidronic Acid and its salts.

Animals (n)	Dose/Concentration	Procedure	Results	Reference
Etidronic Acid				
Male CD rats (n=50)	0, 0.3, 3.0, 30.0, and 100 mg/kg	Administered by gavage for 28 days alone or after administration of milk-derived feed. At the end of the study period, various parameters related to bone growth were evaluated.	There were no clinical signs of toxicity or effects on body weight gain at any dose. The mean weights of bone (femur, tibia, metatarsus and calcaneus) were comparable between groups at all stages of ossification. X-ray studies of the femur and tibia showed minimal enlargement of the epiphyseal area of the tibia at the highest dose, both with and without milk. Using the very sensitive whole-bone morphometric methodology, enlargement of the tibia in the metaphyseal area was observed in the highest-dose group. Histological examination of the tibiae and sternum showed no treatment-related effects. The qualitative evaluation showed regular development of primary spongiosa and of epiphyseal cartilage. NOAEL=30 mg/kg/day	[2,7]
Charles River albino rats (n=15/sex)	0, 3000, 10,000, 30,000 ppm in diet (154, 524, 1583 mg active acid/kg for males and 166, 545 and 1724 mg active acid/kg females, respectively)	Test material in feed for 90 days. Rats observed daily. Blood samples were collected at 45 and 84 days from 10 rats in the control and high-dose groups. All rats were killed and necropsied at the end of the experiment.	1 female in the high-dose group and 1 male in the low-dose group died of unknown causes; 1 female in the control group and 3 males and 7 females in the high-dose group died in association with blood collection. The authors stated that this may indicate increased susceptibility to trauma in the high-dose group. No effects were observed in the low- and mid-dose groups. Body weight gains were decreased in males (84% of controls) and females (92% of controls). Feed intake for males was slightly decreased (92% of controls). Also observed were increased erythrocyte counts (males), decreased hemoglobin concentration (both sexes), decreased hematocrit values (both sexes), and decreased leukocyte counts (females at after 84 days only). Coagulation time and differential leukocyte counts were not affected. Perturbations of hematological parameters were probably related to reduced availability of iron and consequent alterations in iron homeostasis. Bilateral mineralized microconcretions in kidney tubules (3 males affected; no controls) and extramedullary hematopoiesis in the spleen (3 females affected; none in controls) may have been a result of altered calcium homeostasis and related to the test substance. Liver weights were decreased in the high-dose group. No abnormalities of the reproductive organs were attributable to the test substance. It was concluded that the NOAEL≥1724 mg active acid/kg/day in females, and 1583 mg active acid/kg/day in males.	[2,7]
Beagle dogs (n=4/sex)	1000, 3000, 10000 ppm in diet (191, 554, 1746 mg/kg active acid for males and 202, 553, 1620mg/kg active acid for females, respectively)	Test material in feed for 90 days. Dogs were observed daily throughout the exposure period, body weights were measured weekly, and feed consumption was measured throughout exposure, urine and blood (for hematology and clinical chemistry parameters) samples were taken prior to treatment and on Days 42 and 84. At the end of the 90-day exposure period all dogs were killed and necropsied. A selection of organs and tissues were weighed. Histopathology was performed on control and high-dose groups.	There were no treatment-related adverse effects. No abnormalities of the reproductive organs were attributable to the test substance. The NOAEL was ≥1746 mg/kg active acid for males and ≥1620 mg/kg active acid for females.	[2,7]

Table 7. Short-term, subchronic, and chronic oral toxicity animal studies of Etidronic Acid and its salts.

Animals (n)	Dose/Concentration	Procedure	Results	Reference
Disodium Etidronate				
Sprague-Dawley rats (n=10/sex, number in satellite group not specified)	0, 500, 2000, 10,000 ppm (equivalent to 0, 41, 169, 817 mg/kg/day in males and 0, 50, 195, 1000 mg/kg/day in females)	Test substance in feed for 90 days. Rats in the satellite groups were used for laboratory investigations. The rats were treated to an MTD, as indicated by the decrease in bodyweight gain.	1 female in the control group died during blood collection. Clinical signs: severe pallor of skin in high-dose group and slight pallor in mid-dose group at week 6. Body weight was reduced in the high-dose group rats (relative mean weights 92% for males and females). Feed and water consumption were reduced in the high-dose group male rats. Clinical Chemistry: Higher alkaline phosphatase at weeks 5, 7 and 12 in males in the high-dose group; all other parameters were similar to controls. Higher plasma glucose level in females in the high-dose group at weeks 12 and 13; all other parameters were similar to controls. Hematology results showed decreased red cell values (details not provided) and neutrophil and lymphocyte counts were higher at weeks 5 and 7 in the high-dose group. In the mid-dose group, there were decrease in red cell values and higher neutrophil and lymphocyte counts for males at week 7. At week 12, there were reduced red cell parameters for both sexes in the high-dose group and for males in the mid-dose group. Blood smears indicated a retardation of bone marrow development and prolonged anaemia at weeks 5, 7, 12 in both sexes at in the high-dose group. Urinalysis showed an increase in calcium levels in high-dose males at weeks 6 and 12 when expressed in terms of volume of urine but not in absolute terms. The observations are consistent with the perturbation of iron homeostasis (blood smears indicated anaemia and reduction in red cell parameters and pallor). NOAEL=500 ppm, 50 mg/kg LOAEL=2000 ppm, 169 mg/kg	[26]
Charles River CD rats (n=20/sex); (n=20/sex)	0, 0.2%, 1.0% (approximately equivalent to 0, 260 and 1300mg/kg); second study at 0 and 5%	In feed for 91 days	1 female died in 0.2% group; all others survived. Cause and timing of death not specified. Histopathology: No toxic effects. No effects to body weight gain. A slight increase in relative kidney weights in females. In second study (not fully reported), 8 of 10 females and 4 of 20 males died within 1 week. Survivors had stomach ulcers and hyperemia. Inflammation and dilated glands and capillaries confirmed gross findings of gastritis. Generalized erosion of glandular mucosal epithelium was observed. In 5% group there was high total WBC and high neutrophil counts. NOAEL=0.2% (approximately 260 mg/kg/day) LOAEL=1% (approximately 1300 mg/kg/day)	[3,28]
Sprague-Dawley CFY rats (n=40, 10 in satellite group)	0, 500, 2000, 10,000 ppm (equivalent to 0, 19, 78, 384 mg/kg/day in males and 0, 24, 96, 493 mg/kg/day in females)	Test substance in feed for 104 weeks. Rats in the satellite groups were used for laboratory investigations and were killed and necropsied at 26 and 104 weeks.	Mortalities: males: control, 26; 500 ppm, 26; 2000 ppm, 17; and 10000 ppm, 20. Females: controls, 26; 500 ppm, 18; 2000 ppm, 21; and 10,000 ppm, 19. Clinical signs included slight and severe skin pallor observed in the mid- and high-dose groups, which resolved by weeks 52 and 68, respectively. There were no adverse effects in feed and water consumption, body weight change, ophthalmology, clinical chemistry, and urinalysis. Hematological results showed perturbations (in the form of reduced red blood cells, anisocytosis, polychromasia, and hyopchromasis) were observed early in the experiment, but there were no treatment related effects that persisted to 104 weeks. All treatment groups had reduced liver weights at 26 weeks, but not at 104 weeks. The spleen had a lack of iron in a proportion of male rats in the mid- and high-dose groups and in female rats in the high-dose group at 26 weeks but not 104 weeks. No treatment related effects were observed in the low-dose group at 26 or 104 weeks. There were no treatment related non-neoplastic lesions, and those observed were considered normal for this strain and age. Reproductive organs-the weights of testes, seminal vesicles, and prostate of the treated male rats were comparable with those of the control group. Histology of the testes, prostate and seminal vesicles from treated rats had parameters (i.e., spermatogenesis, atrophy, dilation of tubules, or peri-arteritis) within the normal range. There were no histological or weight differences observed in ovaries, uterus, or mammary (gland) of treated compared with	[26]

Table 7. Short-term, subchronic, and chronic oral toxicity animal studies of Etidronic Acid and its salts.

Animals (n)	Dose/Concentration	Procedure	Results	Reference
			untreated female rats. The collective results indicated that the treatment with Disodium Etidronate induced no suppression of gonadal functions. NOAEL=24 mg/kg LOAEL=78 mg/kg Reproductive organs: NOAEL ≥384 mg/kg/day in males and ≥493 mg/kg/day in females.	
Wistar rats (not specified)	0, 3.3 ppm	Test substance in drinking water for 2 years	NOAEL=3.3 ppm	[26]
Male Wistar rats (n=150, 75)	0.184 mg/kg/d with a total intake of 134 mg/kg	Radio-labeled test substance administered in the drinking water for 106 weeks	X-ray studies showed that there was no influence of Disodium Etidronate on morphology and length of bones. Feed consumption and body weight increased slightly in comparison with the control group. There were no treatment related effects observed in the macroscopic and microscopic examinations, blood chemistry (including determination of magnesium, iron and zinc in serum), urinalysis, bone marrow smears, organ weights, and the determination of calcium and phosphor in trachea and tibia.	[3]
Mongrel cats (n=7)	0, 50 and 500 mg/kg/day	Test substance administered in 2 doses each day in chicken bouillon by syringe for 6 weeks. Bone biopsy specimens were collected from the right ulna and samples collected for blood chemistry.	6 of the high-dose group died, 5 by the end of week 2; all of the cats that died had high serum creatinine concentrations. Cats in both of the high- and low-dose groups developed abnormally high serum calcium and phosphorus concentrations compared to controls. No treatment effects could be attributed to the test material in any other hematologic parameters. The only histologic abnormalities seen in the control cats were occasionally small foci of lymphocytic infiltration in the interstitium of the kidneys in the high-dose group. The treated cats had acute and chronic inflammation of the kidneys, involving both interstitial and tubular elements (4 in the low-dose and 6 in the high-dose group). The frequency and severity of the changes were greater in the high-dose group. The relatively severe renal damage was reflected in high serum specific gravity and serum phosphorus and creatinine concentrations in the cats in the high-dose group. The treated cats also had increased widths of osteoid tissue, indicating morphologic osteomalacia.	[30]
Mongrel cats (n=6, 5, 6)	1, 10, 25 mg/kg	Test substance was administered in feed for 6 months.	5 of the 6 cats in the high-dose group had both high and low serum calcium and phosphorus concentrations. In the mid- and high-dose groups, only a third of the cats had hypercalcemia; although the incidence of hyperphosphatemia decreased, the incidence of hypophosphatemia increased. This also was true when the results were expressed in terms of the frequency of abnormal values rather than in terms of the number of cats that behaved abnormally. The final serum calcium values were greater than the initial values in all groups. Pathologic findings were normal in all of the tissues examined, including the kidney. Hematologic findings, other than the calcium and phosphorus concentrations, were normal at 6 months, with the exception of an increase in hemoglobin in most of the cats. The bone sections showed an increase in width of the osteoid tissue and the calcification front at all dose levels.	[30]

Table 7. Short-term, subchronic, and chronic oral toxicity animal studies of Etidronic Acid and its salts.

Animals (n)	Dose/Concentration	Procedure	Results	Reference
Etidronate[a]				
Female Sprague-Dawley rats (n=15)	40, 200, 400, or 1200 mg/kg/day	The rats received a standard or restricted diet (fed 2 h after dosing for 4-4.5 h only) and were administered test substance (40, 200, 400, or 1200 mg/kg/day) by gavage for 4 weeks. Control group received deionized water. The rats were killed and their stomach examined grossly and microscopically for damage.	There were no differences between rats fed *ad libitum* and rats on the restricted feeding regimen. 12 rats died and 5 (1200 mg/kg/day group) were killed before the end of the experiment; these deaths were considered to be treatment-related. 1 rat in the 400 mg/kg/day group was found dead during week 3; this was also considered to be treatment-related. There were no lesions observed in any of the stomachs of the rats in the 40 mg/kg/day group. There was one pyloric erosion/ulcer observed in the 200 mg/kg/day group. The 400 mg/kg/day group was observed to have 2 submucosal acute inflammation lesions; 3 fundic erosive gastritis lesions, and 1 pyloric erosion/ulcer. The 1200 mg/kg/day group was observed to have 5 submucosal acute inflammation lesions; 25 fundic erosive gastritis lesions, and 7 pyloric erosion/ulcers.	[29]

LOAEL=lowest-observed-adverse-effects-level; MTD=maximum tolerated dose; NOAEL= no-observed-adverse-effects-level; WBC=white blood count.
[a] It was not clear if this was the acid or a salt

Table 8. Short-term inhalation studies of sodium etidronate

Animal (n)	Dose/procedure	Results	Reference
Male and female Wistar rats (n=10; 5 in control group)	0 or 0.5 mg/m^3 Nose only for 14 days (duration of daily dosing not specified) followed by a 14-day recovery period.	NOAEC $\geq$ 0.5 mg/m^3 In general, there was very little evidence of respiratory irritation. There were no adverse effects in the cornea or conjunctiva (except in one control male for which corneal and conjunctival keratitis was observed). Cutaneous mucosa of nose, skin of the nose and nostrils had occasional incisions. 7 males in control group and 1 rat in treatment group had inflammation of tongue base. Tracheas and lungs had spontaneous alveolar proteinosis (1 male animal of test group and 1 female control) and occasional calcification diseases (1 male of the control group and 2 females of test group). Majority of female rats of both groups had varying degrees of corticomedullary calcification of the kidney. There was evidence of viral inflammation in pancreas and salivary glands. Base of the tongue, hard and soft palate, proximal sections of esophagus and trachea, and the proximal portion of the epiglottis were normal, erosions were found in the distal third of the trachea (side facing the epiglottis) in the treated group. Eosinophilic leukocytes were observed. The edges were either reactionless epithelium or hyperplastic epithelium. This alteration spread between the surrounding epithelium and the epiglottis, and adjacent salivary glands. Swelling in this area caused by cellular infiltrates and edema. Foreign body giant cell formation was evident after the recovery period. Overall, there was erosion of the large mucosa with central necrosis and fibrin deposition. The cell infiltration was mainly monocytes, eosinophils, granulocytes and macrophages. After the recovery period, the effect on the epithelium was reversed. However, ions of foreign body giant cells and macrophages were observed clearly in subepithelial areas. Alterations were clearly treatment-related.	[3]
Male and female Wistar rats (n=5/sex)	12 mg/m^3 Nose only,4 h/day, 4 times over 14 days	Necropsy of the conjunctivae, cornea, and of the upper part of respiratory system were unremarkable. The lungs had areas of moderate to severe pneumonia. Moderate to severe acute to chronic inflammation was observed in all parts of the respiratory tract. The lungs had an interstitial pneumonia of different grades associated with bronchopneumonia. Specific compound-related alterations were not observed histologically. The association of the test material to inflammation of the respiratory system could not be excluded. The NOAEC was not established.	[3]
Male Wistar rats (not specified)	0 and 94 mg/m^3 Nose only, 5 h/day, 6 times over 14 days followed by a 4-week recovery period.	All treated rats had moderate to high grade subacute or chronic laryngitis immediately after exposure and after the 4-week recovery period. During the recovery period, the laryngitis was milder but the presence of foreign body giant cells indicated an incomplete elimination of the test compound. It was not possible to determine the nature of the residues responsible for the chronic laryngitis. Immediately after treatment, all test rats exhibited moderate inflammation in certain parts of the larynx, which did not completely resolve during the recovery period.	[3]

NOAEC=no-observed-adverse-effects-concentration

Table 9. Genotoxicity assays of Etidronic Acid and its salts.

Concentration/Vehicle	Procedure	Test System	Results	Reference
		In Vitro		
Etidronic Acid				
0.001, 0.01, 0.1, 1, 5, and 10 µL/plate	Ames assay; OECD TG 471 (Bacterial Reverse Mutation Assay)	*S. typhimurium* TA98, TA100, TA1535, TA1537, and TA1538, with and without metabolic activation	Not mutagenic with or without metabolic activation. Cytotoxic at ≥5 µL. Controls had expected results.	[2,3,7]
0.125-0.8 µL/mL	OECD TG 476 (In vitro Mammalian Cell Gene Mutation Test)	Mouse lymphoma L5178Y cells with and without metabolic activation	Inconclusive. No dose response was observed. Without metabolic activation at greatest concentration, mutant frequency was 3 times solvent control value, but same as negative (untreated) control. Without metabolic activation, no dose-response was observed, but a >2-fold increase was observed at lowest dose and a 2-fold increase in comparison with both solvent and untreated controls was seen at greatest dose.	[2,7]
Sodium Etidronate – inference purposes				
Experiment I: 1150, 2300, 4600, 9200, 18,400 µg/mL (equivalent to 556-1784.8 µg/mL active acid), with and without S9 mix. Experiment II: 575, 1150, 2300, 3450, 4600 µg/mL (equivalent to 27.9-446 µg/mL active acid) without metabolic activation; 1312.5, 2625, 5250, 10500, 21000 µg/mL (equivalent to 64-2067 µg/mL active acid) with metabolic activation. 21000 µg/mL (equivalent to 2067 µg/mL active acid) without metabolic activation	OECD TG 476 (In vitro Mammalian Cell Gene Mutation Test); 4 h in Experiment I and 24 h in Experiment II.	Mouse lymphoma L5178Y cells. Solvent /vehicle controls-deionized water	Not genotoxic; cytotoxic at 3450.0 µg/mL after 24 h of exposure.	[3]
Trisodium Etidronate – inference purposes				
2.7, 27, 270, and 2700 µg/plate	Ames assay; OECD TG 471 (Bacterial Reverse Mutation Assay). Positive controls: 67.5 µg/plate (o-nitro-p-phenylenediamine, 270 µg/plate (p-toluol-sulfonic acid hydrazide); solvent controls: 100 µL water+100 µl acetone or 100 µL water+100 µl DMSO.	*S. typhimurium* TA98, TA100, TA1535, TA1537, and TA1538 with and without metabolic activation	Negative with and without metabolic activation	[3]
		In Vivo		
Disodium Etidronate				
18.75 and 150 mg/kg	Mouse micronucleus assay; 2 intraperitoneal doses 24 h apart; sampling time was shorter than recommended in guideline	Male and female CF-1 mice (n=4)	Negative for induction of micronuclei under conditions of test; controls had expected results	[3,26]
Sodium Etidronate – inference purposes				
20, 200, and 1000 mg/kg (0.25 mL) for 5 days; control-water or no treatment.	OECD TG 478 (Genetic Toxicology: Rodent Dominant Lethal Test)	Male C3D2F1/J mice (n=20)	Negative for genotoxic effects.	[3]

DMSO=Dimethyl sulfoxide; OECD TG =Organisation for Economic Co-operation and Development Test Guideline

Table 10. Dermal irritation studies of Etidronic Acid and Its Salts.

Concentration/Dose	Test Population (n)	Procedure	Results	Reference
Etidronic Acid				
100%, 0.5 mL (equivalent to 435 mg active acid)	New Zealand White rabbits (n=6)	24 h then observed at 4, 24, 48, and 72 h and 7 days	No erythema or swelling observed at any time point. PDII=0/8.	[2,7]
100%	Rabbit (strain not specified; n=3)	Clipped, intact and abraded skin under occlusion for 24 h then observed at 4, 24, 48, and 72 h and 7 days	1 h after patch removal, well-defined redness in 1 rabbit with very slight edema. 24 h after removal, there was moderate erythema and slight edema. Inflammation gradually reduced in 2 rabbits with very slight redness 7 days after removal. Tissue necrosis occurred when test substance was in contact with abraded areas for 24 h. Results for intact and abraded skin were pooled. Average PDII at 1h=2.3; 24 h=3.6; 48 h=3.0; 72 h=2.6; 5 d=1.6; 7 days=0.6. Group total=42; Group PDII=3.6. Moderately irritating.	[2,7]
Disodium Etidronate				
100% (moistened with oil)	Rabbit (not specified)	Not specified	Slightly irritating	[26]
20%	Hairless mice (not specified)	Test substance was administered twice daily to same area of skin and gently massaged. Number of applications not specified.	Not irritating	[26]
Tetrasodium Etidronate				
30% aqueous; 0.5 mL (equivalent to 195 mg active acid	New Zealand White rabbits (n=3)	OECD TG 404 (Acute Dermal Irritation/Corrosion); administered to shaved skin under semi-occlusion for 4 h. Rabbits were observed for 72 h.	No signs of dermal irritation except for minimal erythema at 1h. PDII=0 of possible 8	[3,26]
5000 mg/kg; 31% aqueous; equivalent to 1650 active acid	Male and female New Zealand White rabbits (n=5)	Under occlusion for 24 h (see above). Excess test substance was then wiped off. Rabbits were observed for 14 days.	1 female died on day 13 (necropsy revealed signs of intestinal disease, suggestive of mucoid enteritis, not related to test substance). Most animals had severe dermal effects at test site, characterized by necrosis followed by eschar formation and/or exfoliation of eschar tissue, which persisted throughout observation period.	[3]
30%; 0.5 mL	New Zealand White rabbits (n=3)	OECD TG 404; under semi-occlusion then rabbits were observed for 72 h	PDII at 1 h=1; at 24, 48, and 72 h=0	[3]
31% aqueous	Rabbits (n=6)	2 test sites/rabbit under occlusion. Observed at 24.5, 48, 72 h and 7, 10, and 14 days.	Erythema and eschar formation was observed at all observation times with little or no improvement over time; edema was observed at all observation times with some improvement over time. Superficial necrosis, necrosis, desquamation, and/or exfoliation observed at some test sites. PDII=5; Irritating.	[3,26]
31% (equivalent to 151 mg acid)	New Zealand White rabbits (n=6)	2 test sites/rabbit under occlusion for 4 h. Observed at 24.5, 48, 72 h and 7 and 10 days.	Mild erythema and eschar formation was observed in 4 or fewer rabbits at all observation times up to day 7, which resolved by day 10. There was only 1 sign of edema formation at 24 h. No dermal effects were observed at any test site by 48 h. PDII=0.3; Mildly irritating.	[26]
31%; 0.5 mL	New Zealand White rabbits (n=3/sex)	OECD TG 404 with 4 test sites/rabbit over 10% body surface for 4 or 24 h; observed at 1, 24, 48, and 72 h and up to 14 days	Irritation 4 h after exposure was very slight (barely perceptible) or well-defined erythema in 3 of 6 rabbits. All skin reactions resolved by Day 10; no tissue destruction was observed. After 24 h exposure, 5 rabbits exhibited severe erythema with edema, and 5 had epidermal tissue damage at 1 or both sites. All 6 rabbits continued to exhibit irritation at 1 or both sites on Day 14. Slightly irritating.	[3]
Not specified (concentration in product mixture reported as 30%-33%)	New Zealand White rabbits (n=3)	Under occlusion for 24 h; observed for 7 days	24 h PDII = 2.3 Barely perceptible redness in two animals 1 h after exposure. By 24 h there was well-defined erythema, and one instance of very mild edema. This reaction gradually reversed, so that skin was normal by seven days after exposure. Mildly irritating.	[3]

PDII=primary dermal irritation index

Table 11. Animal ocular irritation studies of Etidronic Acid and its salts.

Concentration	Method	Results	Reference
		In Vivo	
Etidronic Acid			
100%; 0.1 mL (equivalent to 87 mg active acid)	Albino New Zealand White rabbit (n=3). Instilled in 1 eye. Eyes of 2 rabbits were rinsed with warm isotonic saline after 24 h exposure; third was rinsed after 4 sec. Eyes were examined at 1, 24, 48, and 72 h, and 5 and 7 d.	After 24 h exposure, there was copious discharge; translucent cornea with iris details were moderately obscured, particularly lower half; moderately severe erythema; and swelling with partial eversion of lids developed within 1h instillation. After 24 and 48 h, corneal opacity increased and conjunctivae became bright red. Lower half of cornea became opaque within 72 h and remained so through day 7 of observation. Because lower portion of iris was invisible and not responding to light, it was concluded that sight had been destroyed. Upper half of eye was only moderately affected because dose was concentrated in conjunctival sac. After 4 sec of exposure, moderate lacrimation, mild edema and erythema, and mild corneal cloudiness with iris details were clearly visible within 1 h. Congestion increased slightly by 24 h, then decreased with only very slight redness remaining after 7 days. It was concluded that Etidronic Acid was corrosive to eyes of rabbits.	2,7
0.1 mL (equivalent to 87 mg active acid)	New Zealand White rabbits (n=6); observed at 10 min,1, 24, 48, and 72 h and 5, 7, 10, 14 and 21 days after exposure	There was immediate severe discomfort with pawing, thrashing and eyes tightly closed. 10 min: Severe erythema (necrosis) and copious discharge. 1 h: Severe erythema (necrosis) and copious discharge. 24 h: Areas of corneal opacity, iris showed no reaction to light, severe erythema, very slight edema in 2 rabbits, copious discharge containing whitish exudate with blood. 2-10 days - slight improvement. 14 days - Ulceration (corneal rupture in one instance). 21 days - Corneal ulceration, slight erythema, and copious discharge in 4 rabbits, 1 rabbit had a score of 0. Overall Draize score was 38.8 out of 110. Study author considered Etidronic Acid to be moderately irritating; ECHA and OECD concluded that test substance was severely irritating/ corrosive to eyes of rabbits.	2,7
0.1 mL	New Zealand White Rabbits (n=5, not clear). Eyes were not washed. Observed at 1, 24, 48 h, and 7 days after exposure. Examined with use of fluorescein.	At instillation there were vocalizations from 3 rabbits. 1 h: Cornea was slightly turbid on about ¼ of total area. Conjunctivae were mostly strongly reddened (single vessels hardly recognizable). There was increased redness up to a diffuse pink flesh of conjunctiva with swelling. Day 7: Turbidity, intensity, and affected area of cornea were increased; in 2 cases eyes were completely turbid (iris not visible). Irises were swollen, but sensitive towards light. For 2 rabbits, iris was invisible on day 2. Conjunctiva had very different reactions from no to very strong swelling. Discharge that started at 1 h post exposure (light to medium) continued to increase. Etidronic acid was moderate to severely irritating to eyes of rabbits.	2
1% and 10%	Rabbit	1% was not irritating and 10% was irritating.	2
Disodium Etidronate			
10% and 100%; 3.00% in a dentifrice; 1.00% in a mouthwash;	Albino New Zealand White rabbit (30 rabbits total, distribution not specified). Test groups: 3 mg Disodium Etidronate, not rinsed; 3 mg Disodium Etidronate, rinsed; 10% Disodium Etidronate solution, not rinsed; 3 mg dentifrice containing disodium etidronate (3%), not rinsed; 3 mg dentifrice containing disodium etidronate (3%), rinsed; 100% mouthwash containing disodium etidronate (1%), not rinsed; Observed at 1, 24, 48, 72 and 96 h.	Maximum average scores and number of days for all rabbits to fully recover: 3 mg Disodium Etidronate, not rinsed-6.6, 2 days; 3 mg Disodium Etidronate, rinsed-5.6, 2-4 days; 10% Disodium Etidronate solution, not rinsed-4.0, 1-2 days; 3 mg dentifrice, not rinsed-21.6, 3-4 days; 3 mg dentifrice, rinsed-9.0, 2-3 days; 100% mouthwash, not rinsed-2.0, 1 day. Dentifrice and mouthwash were moderately irritating.	28
Not specified	Draize test using rabbits	Not irritating	26
20%	Not specified	Not irritating	26
Tetrasodium Etidronate			
30% aqueous; 0.1 mL	Albino New Zealand White Rabbits (n=3); observed at 1, 24, 48 and 72 h.	Cornea and iris total score-0; conjunctival redness-6; conjunctival chemosis-3; conjunctival discharge-3. Maximum group mean score=6.7. Mild irritant.	26

Table 11. Animal ocular irritation studies of Etidronic Acid and its salts.

Concentration	Method	Results	Reference
31% aqueous; 0.1 mL	Albino New Zealand White rabbits (n=6); observed at 1, 24, 48 and 72 h and 7 days.	Initial pain response observed in 2 females. Signs of ulceration in 1 rabbit was resolved by 72 h.; Iris- Dulling of lustre observed in 4 rabbits at 1 h; conjunctival redness-2 at 24 h, .5 at 72 h; conjunctival chemosis-2 at 1 h, .83 at 24 h; discharge-1.3 at 1 h. All effects resolved at 7 days.	[26]
23% aqueous; 0.1 mL	Albino New Zealand White rabbits (n=3); eyes were rinsed 24 h after instillation.	1 h-Draize scores 42, 35, 42 (copious discharge, edema with partial eversion of lids, moderate redness of conjunctivae, diffuse corneal areas with iris details slightly obscured); 24 h- 33, 29, 35 (discharge ceased and swelling reduced); 48 h-28, 19, 28; 72 h-21, 13, 19; 120 h-13, 6, 10 (slight corneal opacity); 168 h-6, 2, 4. Moderate irritant.	[26]

Table 12. Human chronic oral studies of Etidronic Acid and it salts.

Subjects (n)	Dosing/procedure	Results	Reference
Etidronic Acid			
Post-menopausal female human subjects with low bone mass, all under 75 years old (n=28, 22)	Orally administered both Etidronic Acid (400 mg/day) and elemental calcium (500 mg/day) or just elemental calcium (500 mg/day). Etidronic Acid was administered daily for 14 days followed by 76 days of calcium supplementation; this pattern was repeated for 3 years.	Self-reported adverse effects included influenza-like symptoms, joint and bone pains, bronchitis, back pain, heartburn, constipation, cystitis, abdominal cramps, dizziness, and diarrhea. These symptoms were mostly mild and evenly distributed between the two groups. One subject withdrew early in the experiment due to severe diarrhea. Two cases of cancer were diagnosed during the study, but were considered unrelated to test substances.	[22]
Disodium Etidronate			
Human subjects under treatment for osteoporosis (n=24, 37)	Orally administered Disodium Etidronate (400 mg/day) for 14 days followed by calcium supplements for 76 days for 12 cycles over 36 months. Disodium Etidronate was taken at least 2 h before and after eating. Control group was administered 500-1000 mg/day calcium carbonate.	There were no adverse effects reported.	[34]
Human subjects on high-dose corticosteroid therapy (n=59, 58)	In a double-blind, randomized, multicenter, and parallel-group study, oral Disodium Etidronate (400 mg/day) or placebo administered for 14 days, followed by 76 days of oral calcium carbonate (500 mg elemental calcium) cycled over 12 months.	There was no difference between treatment group and control group in number of reported adverse events. During the study, 86% and 88% of subjects in treatment group and control group, respectively, reported an adverse event; these were mostly related to underlying diseases. Upper gastro-intestinal adverse events of moderate severity were reported in 11.9% and 5.2% of patients in treatment and control group, respectively. Proportion of patients with abdominal pain, most frequent adverse event, was 17% and 15.5%, respectively.	[35]
Etidronate[a]			
Human subjects with amyotrophic lateral sclerosis (n=41)	Orally administered etidronate (0 or 400 mg/day) for 2 years. Subjects were examined before and after experiment and every 4 months during experiment.	There were no serious adverse events, including death, overdose, and any other events that were life-threatening or permanently disabling, observed in either group. In the treatment group, 4 subjects experienced abdominal pain, and 1 subject in placebo group experienced anorexia; these symptoms subsided within a week without discontinuing treatment. No subject in treatment group experienced liver or renal dysfunction.	[36]
Human subjects (n=47, 16, 47)	Orally administered etidronate (dose not specified) for at least 1 year (mean 2.6 years). Subjects were divided into groups that ingested doses on waking, during day, or at bedtime, all with at least 2 h before and after ingesting food. Treatments with etidronate were for 14 days followed by calcium supplements for 76 days.	There were no adverse effects reported in any of study groups and there were no differences in effectiveness of treatment on osteoporosis due to ingestion at different times of day.	[19]

Table 12. Human chronic oral studies of Etidronic Acid and it salts.

Subjects (n)	Dosing/procedure	Results	Reference
Human subjects with osteoporosis (n=135, 133, 138)	Orally administered etidronate (400 mg/day) at bedtime for 2 weeks followed by 10 weeks of no treatment. This was repeated 4 times. A second group was treated same way with a lower dose (200 mg/day). Control group was treated with a placebo.	Reported adverse events were gastrointestinal in nature. 1 subject dropped out of the study due to gastrointestinal adverse events. No further details were provided.	[20]
Human Subjects with osteoporosis (n=23, control=24)	Orally administered etidronate (400 mg/day) at bedtime for 2 weeks followed by 10 weeks of no treatment. Controls were treated with calcium lactate (2 g/day).	Adverse effects included gastrointestinal events. None of subjects dropped out due to adverse events.	[37]
Caucasian and Asian female subjects with osteoporosis (n=105, 107, control=104)	Groups 1 and 2 were treated with phosphate or a placebo for 3 days, etidronate (400 mg/day) for 14 days, followed by 74 days of calcium supplementation. Control group was treated with a placebo for first 2 parts of treatment followed by calcium supplementation.	3 subjects in group 1 and 1 subject in group 2 dropped out due to adverse events (details not specified); 2 subjects dropped out in control group. Diarrhea was reported in 7%-9% of all groups.	[38]
Human Subjects with osteoporosis (n=67, control=74)	Orally administered etidronate (400 mg/day) for 2 weeks followed by calcium (400 mg/day) for 71 days. This was repeated 4 times. Control group was treated same way with a placebo followed by calcium.	8 subjects in etidronate group had a total of 9 adverse events that were considered to be causally related to treatment; 8 subjects in placebo group had a total of 18 adverse events. Most adverse events were gastrointestinal in nature and were mild, transient, and similar in frequency in the 2 groups (12 of 74 [16%] in placebo group vs. 13 of 67 [19%] in etidronate group). Gastrointestinal side effects were abdominal pain, diarrhea, constipation, heartburn, and dyspepsia. No subjects withdrew as a consequence of adverse events.	[39]

[a] It was not clear if this was the acid or a salt

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