MINI REVIEW ARTICLE

A Review of the Metabolism of 1,4-Butanediol Diglycidyl Ether–Crosslinked Hyaluronic Acid Dermal Fillers

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BACKGROUND   Cosmetic procedures are growing ever more common, and the use of soft tissue fillers is increasing. Practicing physicians need to be aware of the biological behavior of these products in tissue to enable them to respond to any safety concerns that their patients raise.

OBJECTIVES   To provide an overview of the metabolism of 1,4-butanediol diglycidyl ether (BDDE)-crosslinked hyaluronic acid (HA) dermal fillers and to examine the safety of the resulting byproducts.

METHODS   A review of available evidence was conducted.

RESULTS   After reaction with HA, the epoxide groups of BDDE are neutralized, and only trace amounts of unreacted BDDE remain in the product (<2 parts per million). When crosslinked HA, uncrosslinked HA, and unreacted BDDE degrade, they break down into harmless byproducts or into byproducts that are identical to substances already found in the skin.

CONCLUSION   Clinical and biocompatibility data from longer than 15 years support the favorable clinical safety profile of BDDE-crosslinked HA and its degradation products. Given the strength of the empirical evidence, physicians should be confident in offering these products to their patients.

Cosmetic procedures are growing ever more common, and the use of soft tissue fillers is increasing. The technology and biocompatibility of the products has progressed along with this demand, from the use of silicone injections in the 1940s and 1950s and the first animal-derived collagen fillers of the 1980s through to the introduction of hyaluronic acid (HA) fillers in the 1990s.[1] HA fillers, according to a survey of plastic surgeons conducted by the International Society of Aesthetic Plastic Surgeons (ISAPS), were the second-most-popular nonsurgical procedure of 2010, with more than two million procedures performed.[2] Factors contributing to this popularity include the favorable safety profile of these products, so it is important for physicians to understand what underlies this and to consider the empiric evidence surrounding how these fillers are metabolized and the safety of the byproducts of their degradation.

The three classes of dermal fillers currently in use are[3,4]:

- Absorbable products (temporary; 3–6 months; e.g., HA and collagen fillers).

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- Slowly absorbable products (temporary; 6–24 months; e.g., HA, calcium hydroxyapatite,[5] and l-polylactic acid fillers).
- Nonabsorbable products (permanent; >24 months; e.g., polymethyl methacrylate and silicone fillers).

Hyaluronic acid, the most widely used filler substance currently on the market, has a number of advantages over its predecessors. Crosslinked HA fillers have been used for longer than 15 years[6] and are considered to be generally well tolerated. They have structural properties similar to those of native tissue, excellent biocompatibility, and good tissue integration.[7,8] They have a tunable duration of action spanning the entire range of the temporary filler category (6–24 months), and because of their relatively stable molecular composition, they can be stored without refrigeration for up to 2 years. Because of the hydrophilic nature of HA, these fillers also serve to hydrate the skin, and uniquely among other filler substances, HA can be reversed using hyaluronidase.[9]

In most commercial products, HA is crosslinked to increase its longevity, and the crosslinking agent used has an important effect on the properties of the final product; 1,4-butanediol diglycidyl ether (BDDE) is the crosslinking agent used in the majority of the market-leading HA fillers, and its stability, biodegradability, and long safety record spanning more than 15 years are what make it the industry standard, ahead of other crosslinkers such as divinyl sulfone and 2,7,8-diepoxyoctane.

In the modern era of enhanced patient awareness of the possible complications associated with cosmetic products, it is essential for practicing physicians to be aware of the biological behavior of these products in tissue to enable them to respond to any safety concerns that their patients raise.

Degradation of HA

Hyaluronic acid is a naturally occurring polymer found in the extracellular matrix, the vitreous humor, and the cartilage. The total quantity of HA found in a 70-kg person is approximately 15 g, and its average turnover rate is 5 g/d. Approximately 50% of the total quantity of HA in the human body is concentrated in the skin, and it has a half-life of 24–48 hours.[10]

Hyaluronic acid is a polysaccharide that consists of repeating monomers (glucuronic acid and *N*-acetyl-glucosamine disaccharide units) linked together in a linear fashion through β-1,4 glycosidic bonds. The degradation of HA can be viewed as a depolymerization process that is mediated by glycosidic bond cleavage, which the dissociation of the polymer chains on a macromolecular level may precede (dissolution and diffusion). The depolymerization of HA has been well characterized in the literature and primarily involves two mechanisms: enzymatic degradation and free radical degradation.

Enzymatic Degradation

A large class of enzymes collectively known as hyaluronidases mediate enzymatic degradation. In humans, the most active enzymes of this class are HYAL1 and HYAL2.[10,11] HYAL2 (anchored on the cell membrane) cleaves high-molecular-weight HA (>1 MDa) into 20-kDa fragments. HYAL1 (found in lysosomes) subsequently cleaves these fragments further down to tetrasaccharides, which are then converted to monosaccharides by several enzymes of the hyaluronidase family (e.g., β-glucuronidase, β-*N*-acetylglucosaminidase). Because these degradation products are native to the human body, they join the natural elimination process.[12]

Free Radical Degradation

Regardless of magnitude, disturbance to the tissue environment can activate the body's immune system, leading to a transient inflammatory reaction. Mechanical injury to the tissue caused by the penetration of a needle during filler injection can also lead to such a reaction. Acute tissue inflammation is often linked to a transient increase in free radical activity at the site of the injury. Free radicals are known to

degrade biomaterials through oxidation. Several reports in the literature indicate that free radical-mediated degradation of HA proceeds through cleavage of glycosidic bonds.[13,14] It has not been investigated whether adjunctive aesthetic treatments, such as laser resurfacing, which is known to promote free radical generation,[15] have an effect on the degradation of HA-containing dermal fillers.

Hyaluronic acid catabolism takes place in situ (e.g., in the extracellular matrix), intracellularly, or after transfer to the lymph nodes.[16] Long HA chains (polysaccharides) that enzymes and free radicals degrade in situ yield smaller HA units (oligosaccharides). These smaller HA units are further catabolized intracellularly or in the lymph nodes. Eventually, these units enter the circulatory system, where the liver and kidneys eliminate them.[12,17]

Degradation of BDDE

1,4-Butanediol diglycidyl ether is the crosslinking agent used to stabilize the majority of the HA-based dermal fillers currently available on the market. Its ability to crosslink is attributed to the reactivity of the epoxide groups present at the two ends of the molecule. Under basic (pH > 7) conditions, these epoxide groups preferentially react with the most accessible primary alcohol in the HA backbone, forming an ether bond connection. The superior stability of the ether bond (relative to the ester or amide bond) is one of the reasons that BDDE-crosslinked HA fillers have a clinical duration that can reach or exceed 1 year. In addition, BDDE has a significantly lower toxicity than other ether-bond crosslinking chemistry based agents (e.g., divinyl sulfone), is biodegradable, and has been well studied. All these factors have contributed to BDDE becoming the industry-standard crosslinker.

Although unreacted BDDE has been found to be mutagenic in the Drosophila model organism[18], a definitive carcinogenic effect has not been observed in mice.[19] Despite this, and because of its mutagenic potential (thought to be the result of the reactive

nature of the epoxide groups), the amount of unreacted BDDE in dermal fillers is maintained at trace amounts. For Allergan's range of HA dermal fillers, this is achieved through a complex purification process, which results in a residual level of unreacted BDDE of <2 parts per million (ppm). This would equate to <0.002 mg of BDDE in 1 mL of HA gel. This trace level, which historically was the limit of detection, has been determined to be safe after a Food and Drug Administration (FDA) safety risk assessment.

Over time, unreacted BDDE undergoes degradation through hydrolysis. The most favorable cleavage sites are the ether bonds in the epoxide groups and in the backbone of the molecule. Degradation of BDDE can produce a number of nonreactive by-products (described below).

Hydrolyzed BDDE

Hydrolyzed BDDE is a diol-ether resulting from the hydrolysis of the epoxide groups in BDDE or the hydrolytic cleavage of crosslinked BDDE. Hydrolyzed BDDE has been shown to be nontoxic and nongenotoxic, even at molar concentrations significantly higher than the concentrations used in commercial fillers (J.X. Roca-Martinez, unpublished data). (These concentrations are defined later in this section.)

Although the metabolism of hydrolyzed BDDE is not described in the literature, it is understood to proceed through ether bond cleavage by a family of enzymes called cytochromes P450. These enzymes are involved in the oxidative degradation of organic molecules and can catalyze the cleavage of ether bonds into alcohols.[20] After degradation, two main products can emerge: glycerol and butanediol. Similar to all diol-ethers, hydrolyzed BDDE is also known to be eliminated in urine.[21,22]

1,4-Butanediol

1,4-Butanediol is known to be nonmutagenic, non-sensitizing, and a slight irritant.[23,24] No carcinogenic

potential has been identified by tests performed on its metabolites. Neurotoxic adverse effects were observed in animals with a no observed adverse effect level (NOAEL) of 100 mg/kg per day (determined according to oral administration in mice). The median lethal dose (LD_{50}) of 1,4-butanediol is 1,525 mg/kg (determined according to oral administration in mice).

The metabolic pathway of 1,4-butanediol is summarized in Figure 1.[25] The process yields succinic acid that is further oxidized through the Krebs cycle. A metabolic study performed using ^{14}C-labeled 1,4-butanediol showed no bioaccumulation and that the majority of 1,4-butanediol was rapidly oxidized into carbon dioxide (CO_2).[25]

Glycerol

According to a report by the Organization for Economic Co-operation and Development,[26] glycerol is nonmutagenic, nonteratogenic, nonsensitizing, and a nonirritant, with a NOAEL of 2,000 mg/kg per day and an LD_{50} of 4,090 mg/kg (determined according to oral administration in mice). The safety of glycerol is well understood from its clinical role in reducing intracranial pressure (administered by intravenous infusion at a concentration of 24 mg/kg per day).[27]

Human and animal studies have shown that glycerol kinase phosphorylates glycerol to alpha-glycophosphate in the liver (80–90%) and, to a lesser extent, the kidneys (10–20%). Alpha-glycophosphate is then converted into CO_2 and water through the classic metabolic pathway of glycolysis.[28] Glycerol is rapidly eliminated through these pathways, with an elimination half-life in humans of approximately 30–45 minutes.[29]

The concentration of BDDE-derived byproducts that can be present in commercial fillers is so low (<5 mg/mL, determined using nuclear magnetic resonance spectroscopy), that the corresponding annual dose (<1.7 mg/kg per year, based on a 60-kg patient and a clinical dosage of 20 mL/year) is a small fraction of the NOAEL safety thresholds for daily doses of the BDDE metabolites butanediol (100 mg/kg per day) and glycerol (2,000 mg/kg per day). Therefore, and similar to the trace levels of unreacted BDDE, the presence of modified BDDE byproducts in commercial fillers is highly unlikely to result in toxicity.

Degradation of BDDE-Crosslinked HA

In the previous sections, we reviewed scientific data suggesting that uncrosslinked HA, BDDE, and their metabolites are clinically safe at the concentrations used in HA fillers. After the crosslinking reaction, the chemical structures of BDDE and HA are modified to various degrees. In this section, we review additional preclinical data, which demonstrate that these molecular changes do not pose additional safety risks.

Degradation of Modified BDDE

After the crosslinking reaction, BDDE can be present in different chemical states. As illustrated in Figure 2, BDDE preferentially reacts with the primary alcohol groups in the HA backbone.

The different states in which BDDE can be present in the final product are summarized here:

(A) Fully reacted crosslinker: A BDDE molecule that has reacted with HA on both ends.

(B) Pendant crosslinker: A BDDE molecule that has reacted with HA on one end only.

(C) Deactivated crosslinker: A BDDE molecule that has reacted with H_2O (hydrolyzed BDDE).

(D) Residual crosslinker: A BDDE molecule that has not reacted with HA or H_2O.

As seen earlier, the risk linked to the presence of residual crosslinker is almost completely eliminated through purification of the crosslinked product.
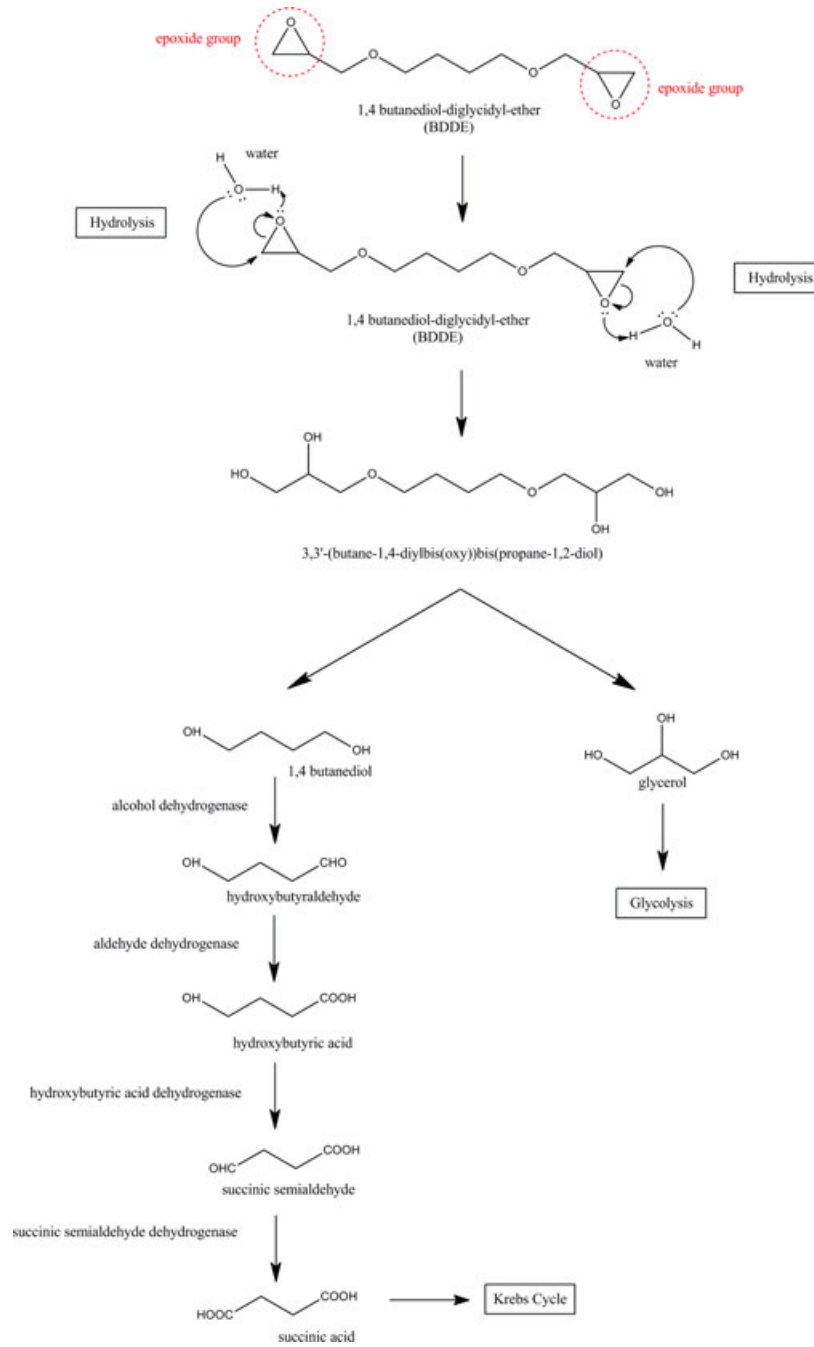
Figure 1. Metabolism of BDDE.

FDA-approved fillers are manufactured with an extremely tight design specification of <2 ppm unreacted BDDE in the finished product.

Because of the absence of the reactive epoxide groups, it is highly unlikely that any of the other chemical states of BDDE result in additional toxicity risks.

Under the basic (pH > 7) conditions of the cross-linking reaction, the majority of the epoxide groups "open" to link to HA through an ether bond or to hydrolyze into an alcohol. It is also likely that any trace amounts of residual crosslinker in the finished product will continue to hydrolyze even under neutral conditions. This epoxide transformation is not
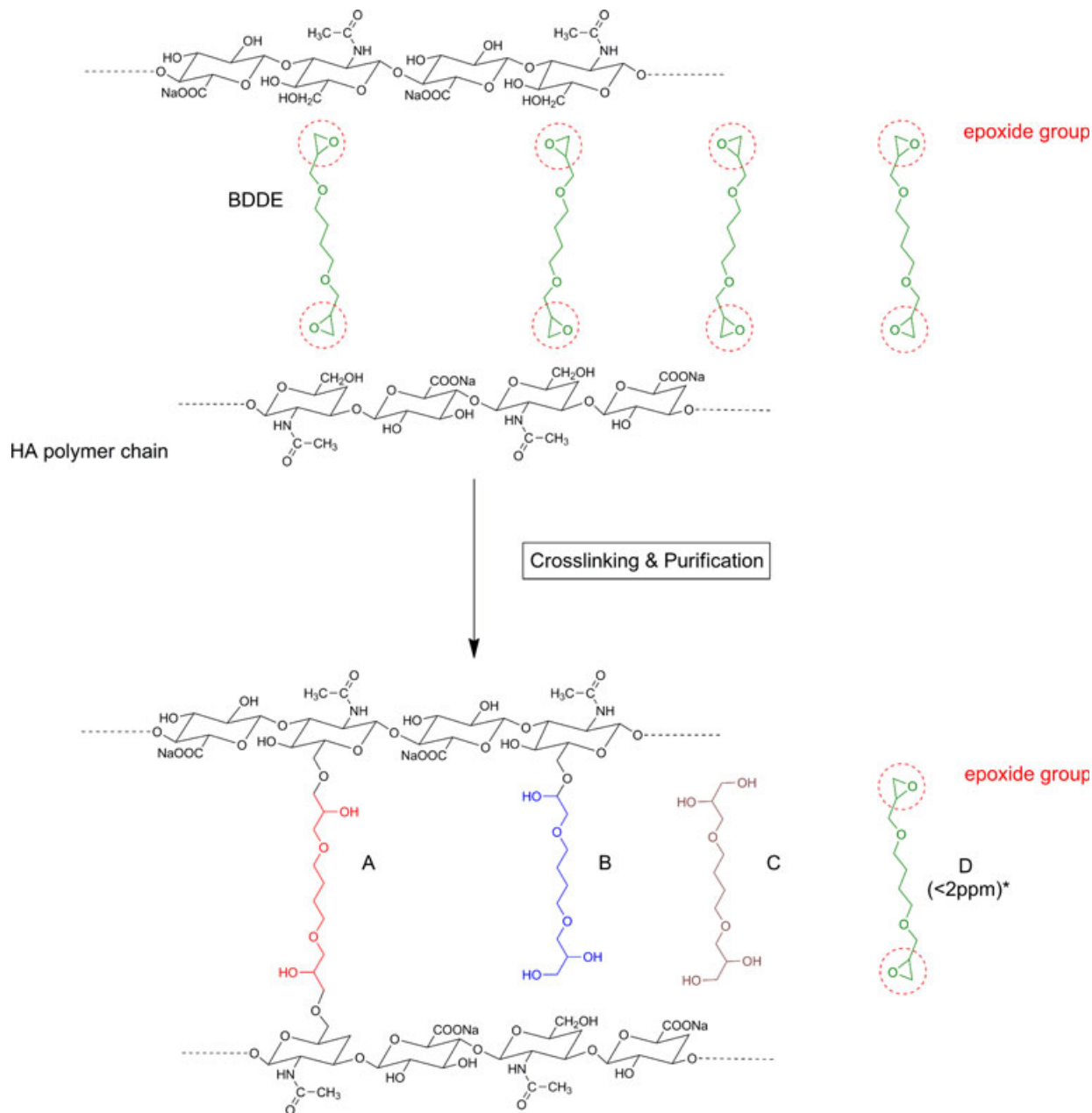
Figure 2. Schematic showing the crosslinking reaction of hyaluronic acid chains with BDDE. The epoxide groups in BDDE preferentially react with the primary hydroxyl groups in the HA backbone, resulting in "fully reacted crosslinker" (A) or "pendant crosslinker" (B). BDDE that has not reacted with HA can be present in its hydrolyzed form "deactivated crosslinker" (C) or its native form "residual crosslinker" (D). *Since the sum of all four forms of BDDE is <5 mg/mL, or 5,000 ppm, the trace levels of the native form D (<2 ppm) represent a minute fraction of that sum.

reversible, and experimental evidence confirms that BDDE cannot be regenerated in the finished product (J.X. Roca-Martinez, unpublished data).

Because the ether bond-based chemistry is preserved in the fully reacted, pendant, and deactivated crosslinkers, it is thought that the in vivo metabolism of these molecules is similar to that of BDDE (by ether-bond cleavage). As discussed earlier, these metabolites (1,4-butanediol and glycerol) have been extensively described in the literature and are considered to be well tolerated in vivo.

Degradation of Modified HA

As described earlier, BDDE reacts mainly with the primary alcohol in the HA backbone (Figure 2). Because the glycosidic bonds in the polysaccharide backbone are maintained after the reaction, it is thought that crosslinked HA is susceptible to the same in vivo degradation mechanisms as uncrosslinked HA (described earlier), and several reports in the literature have confirmed that crosslinked HA is amenable to the same enzymatic, hydrolytic, and oxidative degradation processes that break down native HA in the human body.[30] The hyaluronidase family of enzymes degrades HA by specifically cleaving the glycosidic bonds between glucuronic acid and acetylglucosamine in the HA backbone. Because these bonds remain unaffected after the crosslinking reaction, it is thought that crosslinked HA is also susceptible to enzymatic degradation. Two separate studies using a variety of BDDE-crosslinked HA fillers with different physicochemical properties showed that the BDDE modification does not interfere with the natural enzymatic degradation mechanisms of HA.[31,32] Similarly, it has been shown that the BDDE modification also does not affect the susceptibility of uncrosslinked HA to oxidative degradation (D. Stroumpoulis, unpublished data). More specifically, using size exclusion chromatography, it was shown that crosslinked HA is fully susceptible to free radical-induced oxidative degradation.

Biocompatibility of BDDE-Crosslinked HA fillers

In addition to clinical safety data spanning longer than 15 years, a substantial body of biocompatibility data exists for BDDE-crosslinked HA fillers. As early as the 1980s, reports on acute and chronic biocompatibility of crosslinked HA were published after testing in the vitreous cavity of the eye and viscosupplementation of the osteoarthritic synovial fluid.[33–35] Furthermore, animal studies have successfully provided information on topics such as length of tissue reaction (acute, subchronic, or chronic), type of reaction (systemic vs local tissue toxicity), hypersensitivity, and genotoxicity.

For the majority of the commercially available fillers, much of these data were generated in compliance with regulatory agencies to gain market approval. Using Juvéderm products (Allergan) as an example, Table 1 summarizes a typical biocompatibility profile of crosslinked HA fillers. The tests shown in Table 1 were performed in conformance with international standards (International Standards Organization (ISO) 10993) as mandated by regulatory agencies. Based on these results, no evidence of acute, subchronic, or chronic inflammation; sensitivity; irritation; intracutaneous reactivity; systemic toxicity; hypersensitivity; or genotoxicity were observed for these products.

Furthermore, chronic toxicity and subchronic toxicity data (according to ISO 10993–11) were used to determine what constitutes a safe dose of Juvéderm products in animals. The human equivalent yearly dose was then determined to be equal to 20 mL per 60 kg of body weight (according to ISO 10993–17 Guidelines and the FDA's Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers).

These preclinical results provided the first evidence of the safety of BDDE-crosslinked HA products and paved the way to the first clinical studies.

Clinical Safety of HA Fillers

Hyaluronic acid has a long history of cosmetic use; in 1989, Balasz and Denlinger described the first HA developed as a dermal filler,[1,36] and HA dermal fillers have been used for soft tissue augmentation since the 1990s.[1] The first BDDE-crosslinked HA filler was made available in Europe in 1996, and since then the safety profile of this type of product has been extensively studied. A literature search revealed that, in more than 50 studies conducted over the past 15 years in more than 9,000 patients, BDDE-crosslinked HA fillers have been reported to have a favorable safety profile and be generally well tolerated.

TABLE 1. Biocompatibility Test Results for 1,4-Butanediol Diglycidyl Ether–Containing Products

Biocompatibility Test Objective	Test Standard	Results
Tests for in vitro cytotoxicity	ISO 10993–5	No evidence of cytotoxicity
Tests for irritation and delayed-type hypersensitivity	ISO 10993–10	No evidence of skin irritation (erythema, edema, necrosis) or delayed dermal contact sensitization
Tests for acute systemic toxicity	ISO 10993–11	No evidence of systemic toxicity for up to 4 days after implantation
Tests for subchronic toxicity	ISO 10993–11	No evidence of toxicity or signs of irritation or inflammation for up to 3 months after implantation
Tests for local effects after implantation	ISO 10993–6	No evidence of irritation and minor local tissue reaction for up to 4 weeks and 12 weeks after muscle implantation
Tests for genotoxicity: Ames test	ISO 10993–3	No evidence of genotoxicity
Tests for genotoxicity: Micronucleus Test	ISO 10993–3	No evidence of genotoxicity
Tests for genotoxicity: Chromosomal Aberration Test	ISO 10993–3	No evidence of genotoxicity
Test for chronic toxicity: 12-month study, multiple injections	ISO 10993–11	No evidence of systemic toxicity or local irritation for up to 12 months after implantation

ISO, International Standards Organization.
These data, from multiple regulatory agency-mandated preclinical tests, demonstrate that this BDDE-containing range of products has a favorable safety profile. The products used in all of these tests were from the Juvéderm range.

Summary

Hyaluronic acid is a naturally occurring polymer with a degradation pathway that is well understood. Because the half-life of HA in the skin is only a few days, crosslinking is typically employed to stabilize the HA matrix and provide a clinical duration in soft tissue filling that can reach or exceed 1 year. This duration is predominately achieved using epoxide-based crosslinking chemistry and specifically BDDE as the crosslinking agent. BDDE is the crosslinker used in the majority of the market-leading HA fillers. After reaction with HA, the epoxide groups of BDDE are neutralized, and only trace amounts of unreacted BDDE remain in the product (<2 ppm). These trace amounts, which the FDA has determined to be below the level that is safe after a safety risk assessment, are prone to hydrolysis that ultimately yields CO_2 and water. Crosslinked HA is expected to follow a degradation pathway that is similar to that of uncrosslinked HA and unreacted BDDE, because the crosslinking reaction does not affect the backbone chemistry of these molecules. When the product degrades, it breaks down into harmless byproducts or into byproducts that are identical to substances already found in the skin.

Clinical and biocompatibility data spanning more than 15 years support the favorable clinical safety profile of BDDE-crosslinked HA and its degradation products. Since the launch of the first BDDE-crosslinked HA dermal filler for cosmetic use in 1996, more than 50 studies have been conducted with more than 9,000 patients that have reported on the safety and tolerability of this type of product. This legacy of safety may explain why two million people in 2010 chose to undergo cosmetic procedures with HA dermal fillers, the majority of which use BDDE crosslinking. Given the strength of the empirical evidence, physicians should be confident in offering these products to their patients.

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