

PRECLINICAL SUMMARY
P020023

The device is a clear colorless transparent gel consisting of 20 mg/mL of NASHA (non-animal Stabilized Hyaluronic Acid (HA)). The HA used in the manufacture of the subject device is the same as the one used in another recently FDA-approved medical device (P000029, Deflux™, indicated for treatment of Vesicle Urethral Reflux). The NASHA is obtained from bacterial fermentation (*Streptococcus* strain from *Lancefield group C*).

The following biocompatibility and toxicology tests were conducted on the subject device:

Test	Result
Pyrogenicity (Rabbits):	Did not induce fever
Bacterial Endotoxin (Gel Clot Technique):	<0.5EU/mL
Acute Tox in rabbits (20 mg/ml) 7 days (intradermal):	negative (well tolerated)
Subchron. Tox in rabbits (20 mg/ml) 14 days (intradermal)	negative (well tolerated)
Subchron. Tox in rabbits (20 mg/ml) 21 days (intradermal)	negative (well tolerated)
Cytotoxicity:	negative (No cell lysis)
Ames Test:	non-mutagenic
In Vitro chromosomal Aberration study:	not genotoxic
Mouse Bone Marrow Micronucleus study:	not genotoxic
Sensitization (Magnusson & Kligman):	negative
Muscle Implantation (4 weeks in rabbits):	well tolerated
Muscle Implantation (90 days in rabbits):	no encapsulation

Restylane is formed by crosslinking Hyaluronic Acid (HA) with 1,4-Butanediol Diglycidyl Ether (BDDE). These two components, namely, HA and BDDE are integral parts of the finished device. The release criteria for Restylane include a free BDDE concentration of 2 ppm, which is the detection limit of the instrumentation. The sponsor is unable to detect free BDDE in the final finished device, Restylane. The device is biodegradable, and upon degradation the device will **not** generate HA and BDDE as degradation products. Therefore, under worst case conditions, the maximum amount of free BDDE in Restylane is 2 ppm.

Restylane passed all the biocompatibility tests. The device was shown to be non-mutagenic by Ames Test. BDDE is a sensitizer and has also found to be a mutagen in *Drosophila* (Fouremant *et al*, *Environ Mol Mutagen* 1994; 23(1):51-63). To address the carcinogenicity potential of the residual BDDE (2 ppm) present in the device, the sponsor cited an animal study conducted on BDDE by CIBA-GEIGY.

In the CIBA-GEIGY study, BDDE (0.05%) in acetone was used as a topical application on CFI mice. Beta propiolactone was used as positive control and acetone as negative control. It was observed that there was a statistically significant increase in the incidence of lymphoblastic lymphosarcomas in female mice and there was evidence it was dose-

dependent. The sponsor notes that the number of tumors observed with BDDE was not significantly different from that of the negative control, i.e., acetone, and, therefore, BDDE is not a carcinogen. However, the number of tumors observed in case of BDDE is also equal to that of positive control, *Beta*-propiolactone. The animal study investigators felt the study was not conclusive to say BDDE is a systemic carcinogen.

The sponsor also noted that not only the lymphoblastic lymphosarcomas observed in mice (CIBA-GEIGY study) should be taken into account, rather all types of tumors observed should be taken into consideration in deciding the carcinogenicity of BDDE. FDA agrees with this interpretation. See the attached "Pathology Review of Carcinogenicity" memo, which concludes: "I agree with sponsor's explanation. All the lymphomas should be taken together. To sum the lymphoid tumors of each group as in Table 2 seems reasonable. I analyzed the association in cancer rate between control, 0.05 % BDDE and 0.2 % BDDE by using Chi-square test (SAS. Version 8). There is no association between control and treated groups with different doses of BDDE."

Even though the tumors were counted separately in the animal study and that practice was common at the time of the study, the sponsor provided articles by the NCI and NIH that reported that tumors should not be split by cell origin. For the study in question, when you count the tumors together, there is no increased risk of tumor in the BDDE group.

While the FDA agrees that the animal study did not show a relationship between BDDE and lymphomas, the FDA conducted a carcinogenicity risk assessment assuming a worst-case dose of 2ppm of BBDE present in Restylane. For the details of this cancer risk assessment, please review the attached FDA "Cancer Risk Assessment" review. To summarize this review, assuming the worst-case scenario where Restylane contains 2 ppm of free BDDE, and the tumorigenic dose that was obtained from the CIBA-GEIGY study, the risk assessment is calculated to be 4 in 10^5 (if Total Life Time Dose is considered) and 1 in 10^8 if Daily Dose is considered. In conclusion, even using the data from the animal study in which the tumors were erroneously separated, the calculated risk of cancer is minimal.