Medical Device Testing per ISO 10993 Guidelines
Polyfuze™ Polymer Fusion Label Technology

Extractables Study

The following extractables study was commissioned by Dentagrafix and conducted by a preclinical contract research organization to assess Polymer Fusion Labeling Technology used in a medical device. The study was comprised of two parts:

Part 1:
Typical study programs are designed to first, determine the extractable amount of chemical compounds present in and on any target materials and/or device systems, second, provide quantitative data on those targeted compounds of concern as it relates to specific migration behavior, and lastly, to determine the threshold of both toxicological and physiological limits for those compounds presented during the clinical application. In addition to exaggerated and full term exposure studies, studies which determine the asymptotic extraction behavior of a compound leaching from the target material can be performed and utilized in toxicological risk assessments. Often times aggressive conditions, similar to those utilized in biocompatibility, are utilized to better understand the extraction profile as it relates to a product's overall safety testing program. This can effectively be utilized to full characterize a device's influence on and by the clinical-use environment. Extract solvents typically consist of two or three (2-3) model solvents, considering various dispersion forces and compound solubility factors. The model solvents utilized will typically include 1) Purified Water (polar extraction solution), 2) EtOH (organic solvent), & 3) Hexane (apolar extraction solution). The resulting solutions are analyzed by a variety of analytical techniques, to identify and quantify materials that may have migrated from the product contact material into the solution of interest. One developed Gas Chromatography/Mass Spectrometry (GC/MS) method is utilized to analyze the extract samples for low molecular weight organic compounds which may be derived from monomers, residual solvents, residues from polymer treatments, volatile degradation products, other volatile compounds which may migrate from the material of interest. Another developed Gas Chromatography/Mass Spectrometry (GC/MS) method is utilized to further analyze the extract samples for mid-molecular weight organic compounds which may be derived from process lubricants, plasticizers, anti-oxidants, polymer degradation products, & solvents with a higher boiling point which may migrate from the material of interest. Following, developed Liquid Chromatography method using Mass Spectrometry (GC/MS) detection, as well as, UV Detection is utilized to further analyze the extract samples for higher-molecular weight organic compounds which may be derived from anti-oxidants, fillers, plasticizers, polymerization catalysts, hydrogenation catalysts, anti-slip agents and other polymer additives which may migrate from the material of interest. An Inductively Coupled Plasma method is utilized to determine the Inorganic Compounds present due to the leaching of metal based complexes, fillers, pigments, and catalyst residues.

Part 2:
The Toxicological Risk Assessment (TRA), which will be prepared based on the technical approach described in the ISO 10993-17 guidelines and other regulatory guidance that pertains to the project. The purpose of the TRA will be to evaluate the potential risks associated with leachable chemicals in the Sponsor's test article, based on data from a Chemical Characterization, or Extractable/Leachable (E/L) analysis of the device. The E/L data will be used to estimate a daily exposure to the patient based on the intended clinical use of the test article. Toxicity-based thresholds in the form of Tolerable Exposure (TE) levels will be derived for chemicals of interest and these values will be used to assess the potential hazard from each chemical. Analytes that cannot be identified and chemicals that have not been studied will be included in the risk assessment to the extent possible and we will apply models of predictive toxicology, i.e., quantitative structure-activity relationships (QSAR) models, or programs that assign a hazard ranking based on the chemical's molecular structure as necessary. The objectives, methods, results, and conclusions of the TRA will be described in a written report, which will include a description of the evaluated device, the results of the chemical characterization, and an explanation of the key assumptions in estimating patient exposures and risk.

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Test Results

L929 NEUTRAL RED UPTAKE TEST: ASSESSED CYTOTOXICITY POTENTIAL

TEST ARTICLE:
Dentagrafix plastic sheets that have been marked using Polymer Fusion Labeling Technology then thermoformed into final finished devices.

RESULT:
The optical density (OD) of the cells exposed to the test and control articles, as well as the calculated viability percentage, are presented in the following table. The average OD of the peripheral wells, that did not contain any cells, was calculated and subtracted from the average OD of the wells dosed with the control and test articles, for added specificity of the viability %.

CONCLUSION:
Based on the criteria of the protocol and the ISO 10993-5 guidelines, the test article meets the requirements of the test and is not considered to have a cytotoxic potential.

KLIGMAN MAXIMIZATION TEST: EVALUATED SENSITIZATION RISK

TEST ARTICLE:
Dentagrafix plastic sheets that have been marked using Polymer Fusion Labeling Technology then thermoformed into final finished devices.

RESULT:
All animals were within the specified range of body weights (300-500 g) at the initiation of the study (Day 0). No systemic signs of toxicity were observed in treated or control animals. None of the treated (NaCl or CSO extracts) or negative control animals exhibited any reaction at the challenge (0% sensitized). The positive control article elicited discrete (Grade 1) reactions in all animals (100% sensitized).

CONCLUSION:
The USP 0.9% Sodium Chloride for Injection (NaCi) and Cottonseed Oil (CSO) extracts of the test article, Dentagrafix plastic sheets that have been thermoformed into final finished devices., elicited no reaction at the challenge (0% sensitization), following an induction phase. Therefore, as defined by the grading scale of the USP, the test article is classified as a non-sensitizer.

Based on the criteria of the protocol and these results, the test article meets the requirements of the ISO 10993-10 guidelines.

DIRECT PRIMARY ORAL (BUCCAL) IRRITATION TEST: ACUTE EXPOSURES

TEST ARTICLE:
Dentagrafix plastic sheets that have been marked using Polymer Fusion Labeling Technology then thermoformed into final finished devices.

RESULT:
There were no significant clinical signs of toxicity in any of the test or control animals. All three test animals lost weight. Weight loss was not unexpected with the hourly handling of the animals from the previous day of dosing. There were no adverse signs of erythema or edema in any of the test or control sites. No lesions were noted in any of the animals at necropsy. The Irritation Index for the test article was calculated according to the evaluation criteria stated in the protocol and was determined to be 0.3 indicating that the test article was a non-irritant.

CONCLUSION:
The test article was evaluated for its potential to produce primary buccal irritation following exposure (minimum of five minutes per hour for four hours) to the cheek pouches of Golden Syrian Hamsters.

Based on the criteria of the protocol, the test article is considered to be a non-irritant to the buccal tissues of Golden Syrian Hamsters.

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Test Results

SYSTEMIC INJECTION TEST: TESTED FOR SYSTEMIC TOXICITY

TEST ARTICLE:
Dentagrafix plastic sheets that have been marked using Polymer Fusion Labeling Technology then thermoformed into final finished devices.

RESULT:
One test animal lost a biologically significant amount of weight (more than 10%). Eight test animals and six control animals lost a biologically insignificant amount of weight (less than 9%). All of the other test and control animals increased in weight. None of the test or control animals exhibited overt signs of toxicity at any of the observation points.

CONCLUSION:
The USP 0.9% Sodium Chloride for Injection (NaCl) and Cottonseed Oil (CSO) extracts of the test article, Dentagrafix plastic sheets that have been thermoformed into final finished devices., did not induce a significantly greater biological reaction than the control extracts following a single dose to Albino Swiss mice.

Based on the criteria of the protocol, the test article meets the requirements of the ISO 10993–11 guidelines.

RISK ASSESSMENT OF EXTRACTABLE AND LEACHABLE COMPOUNDS

TEST ARTICLE:
Dentagrafix plastic sheets that have been marked using Polymer Fusion Labeling Technology then thermoformed into final finished devices.

CONCLUSION:
The concentrations of extractable elements and organic compounds from the test article were evaluated with respect to potential toxicological risks to patients. The total amounts of leachable analytes as determined using extractions with three solutions: 1) Purified Water, 2) Isopropyl Alcohol (IPA), and 3) Hexane under exhaustive extraction conditions 50 ± 2°C for 96 ± 2 hours, were used to represent a quantity of each compound that could theoretically leach from the test article during its clinical use. Resulting estimates of exposure were compared to derived Tolerable Exposure (TE) levels, which are based on an assumption of daily exposure.

The estimated exposures for detected metals in sample extracts are below their tolerable exposure limit, with a margin of safety (MOS) above 1.0, indicating no potential toxicological risk from the exposure of these compounds.

It should be noted that the aggressive extractions are likely to over-predict the clinical rate of leaching as the high extraction temperature may have significantly increased the extractable levels [81. It is noted that aggressive extraction was conducted for the test article at 50°C while clinical leaching would occur at a body temperature of 37°C. It is also noted that these compounds are detected in the extraction with IPA and Hexane, which may induce higher levels of leachables than the leachable levels would occur for saliva (saliva is 99% water and 1% protein and salts) which is more polar in nature. Therefore, the exposure to this organic substance at the detected level may be unlikely to be associated with unacceptable risks.

Sponsor has also conducted a comprehensive battery of biocompatibility studies as required per SO 10993-1 guidance and demonstrated that the test article was biocompatible.

The results of the study showed that the device was non-sensitizer, non-irritant, non-cytotoxic in vitro and devoid of systemic toxicity in vivo. As there were several compounds with genotoxic alerts in QSAR analysis, toxicology profile derived for some of them shows no genotoxic potential. The clinical use of the device would be buccal cavity and hence analytes from Hexane or IPA are less likely to pose a toxicological concern since saliva is more polar in nature.