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Expert statement in response to the inquiry regarding the assessment of the potential toxicological risk of Urine Catheter Valve KV 200 EH KP

Customer:	Max Stäubli AG Spätzstrasse 14 8812 Horgen Switzerland
Project No:	163498
Inquiry by:	Mr. Werner Maag
Statement issued:	26 September 2016
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Product Description

The test item Urine Catheter Valve KV 200 EH KP is a connector between the urinary bag and the catheter tube and is exclusively used for the urine flow from the patient into the urine bag.

Test Item:

Name:	Urine Catheter Valve KV 200 EH KP
Batch No.:	134747
Type of Material:	synthetic polymer (POM (Polyoxymethylen)), synthetic Elastomer
Calculated Surface as provided by the Sponsor:	140.7 cm^2/test item
Sterility:	sterile
Sterilisation Method:	EO
Storage Conditions:	at room temperature, protected from light

Body Contact

The medical device Urine Catheter Valve KV 200 EH KP has permanent contact (>30 days) to intact skin.

Biocompatibility according to ISO 10993-1

According to the ISO 10993-1 following biological end points should be considered:

- Cytotoxicity
- Sensitisation
- Irritation

Submitted Documents

Report:

- *In vitro* Cytotoxicity Assay: Cell Growth Analysis via BCA-Staining with an Extract of Urine Catheter Valve KV 200 EH KP (Eurofins Munich Project 151501)
- Characterisation of Extractable Organic Compounds by GC-MS/FID after Liquid Extraction Urine Catheter Valve KV 200 EH KP (Eurofins Munich Project 151502)
- *In vitro* Cytotoxicity Assay: Cell Growth Analysis via BCA-Staining with an Extract of Kupplungsstück Trichter (Silikonkautschuk) (Eurofins Munich Project 152499A)
- *In vitro* Cytotoxicity Assay: Cell Growth Analysis via BCA-Staining with an Extract of Kupplungsstück Gehäuse (POM) (Eurofins Munich Project 152499B)
- *In vitro* Cytotoxicity Assay: Cell Growth Analysis via BCA-Staining with an Extract of the single components:
    - Verschlusskappe braun (Eurofins Munich Project 154820A)
    - Schiebehülse braun (Eurofins Munich Project 154820B)
    - Führungsnippel braun (Eurofins Munich Project 154820C)
    - Knickschlauch (Eurofins Munich Project 154820D)
    - Clips orange (Eurofins Munich Project 154820E)

  - Trichter leer (Eurofins Munich Project 154820F)
  - Kupplung (Eurofins Munich Project 154820G)
  - O-Ring grün (Eurofins Munich Project 154820H)
  - Feder rostfrei zu KV (Eurofins Munich Project 154820I)
- *In vitro* Cytotoxicity Assay: Cell Growth Analysis via BCA-Staining with an Extract of POM raw materials and dyes:
  - Führungsnippel Delrin 500P NC010 natur (Eurofins Munich Project 160639A)
  - Führungsnippel Delrin 500P NC010 UN-TR-MB-89612 (Eurofins Munich Project 160639B)
  - Masterbatch UN-TR-MB-89612 beige (Eurofins Munich Project 160639C)
  - Masterbatch UN2339 orange (Eurofins Munich Project 160639D)
  - Delrin 500P NC010 natur (Eurofins Munich Project 160639E)
  - Delrin SC655 NC010 natur (Eurofins Munich Project 160639F)

Performed Studies

Test Material: Urine Catheter Valve KV 200 EH KP		
Test	**Testing conditions**	**Result**
In vitro Cytotoxicity Assay: Cell Growth Analysis via BCA-Staining according to ISO 10993-5 (151501)	The extraction was carried out in compliance with ISO 10993-5 and 10993-12. The test item was extracted under agitation for 24 ± 2 h in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 ± 1 °C. The final surface/volume ratio in the assay was 3 cm^2/mL which corresponds to 100% extract concentration. The extraction procedure did not reveal any abnormalities in the extraction medium or the test item. No changes regarding clarity, color and presence or absence of foreign material occurred in the extraction medium. The test item was tested as provided by the sponsor.	leachable cytotoxic substances were released from the test item (see Table 1)
Characterization of extractable organic compounds by GC-MS/FID according ISO 10993-18 (151502)	The extraction was carried out in compliance with ISO 10993-12. The test item was extracted under agitation under light protection for 72 ± 2 h at 37 ± 1 °C in water, isopropanol and synthetic urine, respectively. The surface/volume ratio in the assay was 3 cm^2/mL. The water, isopropanol and synthetic urine extraction procedure did not reveal any abnormalities in the extraction medium or the test item. No turbidity, no colour changes and no particles occurred in the extraction media.	Extractable substances were detected (see Table 2)
In vitro Cytotoxicity Assay: Cell Growth Analysis via BCA-Staining according to ISO 10993-5 (152499)	The extraction was carried out in compliance with ISO 10993-5 and 10993-12. The test item was extracted under agitation for 24 ± 2 h in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 ± 1 °C. The final surface/volume ratio in the assay was 3 cm^2/mL which corresponds to 100% extract concentration. The extraction procedure did not reveal any abnormalities in the extraction medium or the test item. No changes regarding clarity, color and presence or absence of foreign material occurred in the extraction medium. The test item was tested as provided by the sponsor.	leachable cytotoxic substances were released from the test item (see Table 1)
In vitro Cytotoxicity Assay: Cell Growth Analysis via BCA-Staining according to ISO 10993-5 (154820)	The extraction was carried out in compliance with ISO 10993-5 and 10993-12. The test item was extracted under agitation for 24 ± 2 h in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 ± 1 °C. The final surface/volume ratio in the assay was 3 cm^2/mL which corresponds to 100% extract concentration. The extraction procedure did not reveal any abnormalities in the extraction medium or the test item. No changes regarding clarity, color and presence or absence of foreign material occurred in the extraction medium. The test items were tested as provided by the sponsor.	leachable cytotoxic substances were released from the test item (see Table 1)

-Table continued-

Test	Testing conditions	Result
In vitro Cytotoxicity Assay: Cell Growth Analysis via BCA-Staining according to ISO 10993-5 (160639)	The extraction was carried out in compliance with ISO 10993-5 and 10993-12. The test item was extracted under agitation for 24 ± 2 h in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 ± 1 °C. The final surface/volume ratio in the assay was 3 cm^2/mL (160639A+B) or 0.2 g/mL (160639C-F) which corresponds to 100% extract concentration. The extraction procedure did not reveal any abnormalities in the extraction medium or the test item. No changes regarding clarity, color and presence or absence of foreign material occurred in the extraction medium. The test items were tested as provided by the sponsor.	leachable cytotoxic substances were released from the test item (see Table 1)

Databases

For this assessment, the toxicological data provided in selected toxicological fact databases were taken into account. No further scientific or toxicological evaluation is provided in this document and must be regarded preliminary and not exhaustive.
The toxicological assessment of the leachable under investigation was based upon the following fact databases:

- Various international databases provided by the United States National Library of Medicine TOXNET (www.toxnet.nlm.nih.gov):
  - Hazardous Substances Database (HSDB)
  - Chemical Carcinogenesis Research Information System (CCRIS)
  - Genetic Toxicology Data Bank (GENTOX)

TOXNET:
TOXNET (TOXicology Data NETwork) is a cluster of 16 databases covering toxicology, hazardous chemicals, environmental health and related areas.
For the evaluation performed in this document, the following fact databases were particularly investigated:

HSDB® (Hazardous Substances Data Bank)
HSDB is a toxicology data file. It focuses on the toxicology of potentially hazardous chemicals. It is enhanced with information on Human exposure, industrial hygiene, emergency handling procedures, environmental fate, regulatory requirements, and related areas. All data are referenced and derived from a core set of books, government documents, technical reports and selected primary journal literature. HSDB is peer-reviewed by the Scientific Review Panel (SRP), a committee of experts in the major subject areas within the data banks's scope. HSDB is organized into individual chemical records, and contains over 5000 such records.

CCRIS (Chemical Carcinogenesis Research Information System)
CCRIS is a toxicology data file. It is a scientifically evaluated and fully referenced data bank, developed and maintained by the National cancer Institute (NCI). It contains over 9000 chemical records with carcinogenicity, mutagenicity, tumor promotion, and tumor inhibition test results. Data are derived from studies cited in primary journals, current awareness tools, NCI reports, and other special sources. Test results have been reviewed by expert in carcinogenesis and mutagenesis.

GENETOX (Genetic Toxicology)
GENETOX is a toxicology data file. It is created by the U.S. Environmental Protection Agency (EPA) and contains genetic toxicology 8 mutagenicity) test data, resulting from expert peer review of the open scientific literature, on over 300 chemicals. The GENETOX program was established to select assay systems for evaluation, review data in the scientific literature, and recommend proper testing protocols and evaluation procedures for these systems.

Results

In the *in vitro* cytotoxicity assay the finished product Urine Catheter Valve KV 200 EH KP showed a clear cytotoxic effect (92% growth inhibition at 100% extract concentration). Since the test item consists mainly of the two raw materials silicone rubber and polyoxymethylene (POM), in a further study these two components were tested separately (study no. 152499). Both test items showed clear cytotoxic effects (90% and 91% growth inhibition at 100% extract concentration, respectively). Therefore, all single components were tested separately in a further cytotoxicity assay (study no. 154820), showing cytotoxic effects for POM components or colored (brown or orange) components, whereas silicone components showed no clear cytotoxic effects (see Table 1). As a consequence of these results the raw material polyoxymethylen and the dyes were further investigated. Within a further cytotoxicity test (study no. 160639) the POM without dye as raw material in two different qualities ("normal": 500P NC010 and "medicinal": SC655 NC010), both dyes (master batches) and two die casting manufactured components ("Führungsnippel" made of 500P NC010) with two colors (nature and colored). Within this study the dyes (master batches) showed no clear cytotoxic effects, whereas the both die casting manufactured components showed cytotoxic effects. The tested POM without dye as raw material in two different qualities ("normal": 500P NC010 and "medicinal": SC655 NC010) showed no cytotoxic effect for the normal and a cytotoxic effect for the medicinal quality POM. Finally, a clear reason for the cytotoxic effects could not be identified.

Table 1 shows a summary of the cytotoxicity results.

Table 1: Overview of results of cytotoxicity experiments

Study No.	Test Item	Result
151501	Urine Catheter Valve KV 200 EH KP	92% growth inhibition at 100% extract conc.
152499A	Kupplungsstück Trichter (Silikonkautschuk)	90% growth inhibition at 100% extract conc.
152499B	Kupplungsstück Gehäuse (POM)	91% growth inhibition at 100% extract conc.
154820A	Verschlusskappe braun	96% growth inhibition at 100% extract conc.
154820B	Schiebehülse braun	100% growth inhibition at 100% extract conc.
154820C	Führungsnippel braun	97% growth inhibition at 100% extract conc.
154820D	Knickschlauch	27% growth inhibition at 100% extract conc.
154820E	Clips orange	91% growth inhibition at 100% extract conc.
154820F	Trichter leer (raw material: silicone)	25% growth inhibition at 100% extract conc.
154820G	Kupplung (raw material: POM)	94% growth inhibition at 100% extract conc.
154820H	O-Ring grün	27% growth inhibition at 100% extract conc.
154820I	Feder rostfrei zu KV (raw material: metal)	56% growth inhibition at 100% extract conc.
160639A	Führungsnippel Delrin 500P NC010 natur	97% growth inhibition at 100% extract conc.
160639B	Führungsnippel Delrin 500P NC010 UN-TR-MB-89612	97% growth inhibition at 100% extract conc.
160639C	Masterbatch UN-TR-MB-89612 beige	21% growth inhibition at 100% extract conc.
160639D	Masterbatch UN2339 orange	17% growth inhibition at 100% extract conc.
160639E	Delrin 500P NC010 natur	24% growth inhibition at 100% extract conc.
160639F	Delrin SC655 NC010 natur	98% growth inhibition at 100% extract conc.

Conc. = concentration

Furthermore, the test item was characterized for extractable organic compounds by GC-MS/FID after liquid extraction. In the GC-MS/FID study under the given conditions one organic substance was detected in the water extract. In the isopropanol extract 326.6 µg extractable substances/cm^2 test material surface area were detected. In the synthetic urine extract no extractable substances were detected above or equal to the LOQ (LOQ: 5.0 µg organic substances/cm^2 test material surface area). Table 2 shows the extractable substances, which were detected.

Table 2: Characterisation and quantification of organic substances in the water, isopropanol and synthetic urine extract by GC-MS/FID

Substance [CAS]	Amount in extraction media [µg/cm^2] / [mg/test sample]			ID Level[1]
	Water Extract	Isopropanol Extract	Synthetic Urine Extract	
Probably dimethyl silanediol [1066-42-8]	7.6/ 1.1	--	--	B
Aliphatic oxygen containing compound	--	5.5/ 0.77	--	D
Compound with carbonyl group and ether bond	--	7.2/ 1.0	--	D
Linear or cyclic siloxanes	--	313.9/ 44.2	--	C
Total amount Σ [µg/cm^2]	**7.6**	**326.6**	**--**	**--**

ID Level[1]: Identification Level
A Unambiguous identification of the compound´s CAS-number from the NIST library. MF/RMF >800.
B Identification of the compound´s CAS-number from the NIST library. MF/RMF <800, results backed by experience.
C Identification of the compound´s substance class without designation of a CAS-number.
D Identification of the compound´s rough structure description without designation of a CAS-number.
E Unknown compound detected in FID only.

Dimethylsilanediol [1066-42-8]

Dimethylsilanediol was detected only in the water extract with 7.6 µg/cm^2 corresponding to 1.1 mg/ test item.

Octamethylcyclotetrasiloxane (= Dimethylsilanediol) has widespread use in a variety of applications including fermentation processes, instant coffee production, paper coatings and sizing, diet soft drinks, waste yeast tanks, food washing solutions, adhesives, textiles, de-asphalting, boiler treatments, detergents, cleaning solutions, surfactants, cosmetic products, and polishes [USEPA/OTS; Tech Sup Doc Octamethylcyclotetrasiloxane (1985)].

The HSDB database provides two citations of human exposure studies. In the first citation a repeated insult patch test with 50 human subjects resulting in not sensitizing was performed [European Chemicals Bureau; IUCLID Dataset, Octamethyltetrasiloxane (556-67-2) (2000 CD-ROM edition)]. In the second study normal volunteers were exposed to 10 ppm D4 or air for 1 h via a mouthpiece using a double-blind, crossover study design. The authors analyzed pro-inflammatory cytokines and acute-phase reactants in peripheral blood, markers for a systemic inflammatory response, as surrogate markers for adjuvancy. These tests were repeated when the volunteers were re-exposed to D4 approximately 3 months after the initial exposure. Blood was obtained prior to exposure, immediately post-exposure, and 6 and 24 h post-exposure. In these short-term, controlled human exposures, no immunotoxic or proinflammatory effects of respiratory exposure to D4 were found [Looney RJ et al; Toxicol Sci 44 (2): 214-20 (1998)].

Furthermore, there are two citations on mutagenic information of dimethylsilanediol in the HSDB database. Both mutagenicity studies were negative

Linear or cyclic siloxanes

For the substances described as linear or cyclic siloxanes the only an identification of the compound´s substance class without designation of a CAS-number could be described.

Siloxanes are widely used in many different fields of application, such as cosmetics, personal care products, drugs, food products as well as implantable devices. Generally, siloxanes have been regarded as safe due to the widespread exposure during the last decades, even though only few siloxanes are adequately tested on health effects.

In the HSDB database one citation can be found that clinical studies have shown silicones to be nonirritant when applied to skin [Osol, A. and J.E. Hoover, et al. (eds.). Remington's Pharmaceutical Sciences. 15th ed. Easton, Pennsylvania: Mack Publishing Co., 1975., p. 1530].

Nevertheless, it can be stated that only comparably small amount of these substances were detected in the isopropanol extract that represents an exaggerated extraction of the test item.

Aliphatic oxygen containing compound etc.

For the substances described as aliphatic oxygen containing compound and compound with carbonyl group and ether bond only the compound´s rough structure could be described without designation of a CAS-number. Due to the identification level, an evaluation of the toxicity of the substances cannot be performed. Nevertheless, it can be stated that only comparably small amount of these substances were detected in the isopropanol extract that represents an exaggerated extraction of the test item.

Statement

Based upon the information provided by the sponsor, Max Stäubli AG, the test item Urine Catheter Valve KV 200 EH KP is a connector between the urinary bag and the catheter tube and is exclusively used for the urine flow from the patient into the urine bag. The backflow of urine into the patient is not possible due to a special barring. Furthermore, there is no access to the catheter from the valve. Since approximately 30 years the Urine Catheter Valve KV 200 EH KP is available on the market.

In the *in vitro* cytotoxicity assay the finished product Urine Catheter Valve KV 200 EH KP showed a clear cytotoxic effect. Further cytotoxicity tests of the single components and also the evaluation of the raw materials and dyes resulted in different cytotoxic effects, ranging from no to clear cytotoxicity, without identifying a clear reason for the cytotoxic effects.

In vitro cytotoxicity tests are used as the first step in biocompatibility evaluation of medical devices. Cytotoxicity tests are very sensitive due to the isolation of the cells in cultures, and the absence of the protective mechanisms in the body. It is difficult to predict the *in vivo* toxicity of such data, and no material can be considered biocompatible based only on cell culture tests. However, the skin forms an effective barrier between the organism and the environment preventing invasion of pathogens and fending off chemical and physical assaults, as well as the unregulated loss of water and solutes. Taking this barrier function into account, positive cytotoxicity test results may not always reflect the real toxic potential of a medical device in an *in vivo* situation.

Furthermore, the test item was characterized for extractable organic compounds by GC-MS/FID after liquid extraction. In the GC-MS/FID study under the given conditions one organic substance was detected in the water extract. In the isopropanol extract 326.6 µg extractable substances/cm^2 test material surface area were detected. In the synthetic urine extract no extractable substances were detected above or equal to the LOQ. Water and synthetic urine is typically used for simulating the physiological conditions during the product-use and the procedure with isopropanol represents an exaggerated extraction of the test item. Dimethylsilanediol, which was found in the water extract, was identified as nonsensitizer [HSDB] and no citations about irritation could be found.

The test item consists mainly of the two raw materials silicone rubber and polyoxymethylene (POM). In the HSDB database citations can be found that silicones are non-irritant when applied to skin and polyoxymethylene can be irritating to skin, eyes, and respiratory system.

The test item is a connector between the urinary bag and the catheter tube and is exclusively used for the urine flow from the patient away into the urinary bag. The backflow of urine into the patient is not possible due to a special barring and there is no access from the valve to the catheter. Furthermore, the test item is exclusively used on intact skin which functions as barrier.

Finally, the Urine Catheter Valve KV 200 EH KP is on the market since approximately 30 year with about 1 million sold pieces. During this period no reclamations concerning harmful effects, like irritations on the patients' skin, were known, based on information given by the sponsor.

The requirement of further *in vivo* testing is not necessary. Based on data base and considering the animal protection regulation according to EN ISO 10993-2, animal experimental test were not justifiable.

It shall be noted that the above brief toxicological statement makes no claim to be complete or to be finally conclusive. The suitability of Urine Catheter Valve KV 200 EH KP in a given end-use environment is dependent upon various conditions, e.g. design, manufacturing, sterilization, reprocessing steps or any other changes in the material. It is the sole responsibility of the manufacturer of the final end-use product to determine the biocompatibility.

The author does not assume any liability for potential biological hazards or adverse clinical reactions that may be caused by the materials and/or medical devices used.

Planegg, September 26, 2016

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