CUSTOMER SERVICE REPORT
CS0119

Technical Documentation
FM480

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Edition 2, October 2009

**** The information in this report has been prepared with utmost care and, to the best of our knowledge, contains accurate information. However, the validity of this information and its application in any specific commercial or other case is subject to confirmation by Datwyler in a formal contract. ****

1. Table of Contents

2. Description

FM480 is a novel Datwyler compound based on a blend of bromobutyl and of SBR (styrene butadiene rubber) elastomer.

FM480 is intended to fulfill the latest requirements that are imposed on prefillable syringe and cartridge components.

Compound FM480 is a chemically clean material that is low in extractables. The low extractable profile allows this material to be used in combination with a large variety of drugs. Obviously, FM480 is in compliance with the chemical requirements of all pharmacopoeias on extractables from elastomeric materials.

Its hardness of 53° Shore A makes it extremely suitable for use as a plunger compound for prefillable syringes and cartridges. FM480 displays an appropriate gliding behaviour with adequate activation and gliding forces.

Unlike existing plunger materials, it has the unique property of exhibiting a low permanent deformation when being irradiated in compressed condition ('irradiation set'). Therefore FM480 easily allows gamma irradiation in bulk packaging as is encountered in RTP (Rapid Transfer Port) applications.

Tip caps in FM480 assembled on prefillable syringe barrel tips are demonstrated to be permeable enough for ethylene oxide to allow sterilization of intentionally contaminated barrel tips. FM480 thus offers a chemically superior alternative to polyisoprene formulations that are most often used for tip cap applications.

Components manufactured in FM 480 are also available as Ready-for-Sterilization closures (RfS) or as Ready-to-Use closures (RTU).

FM480 is registered within Datwyler Pharma Packaging's USA Inc FDA Drug Master File (#10953) and with the Health Protection Branch in Canada (#1994-027).

Note: FM480 refers to the type of compound. The extension "/0", "/1", … refers to the colour of the said compound.
Differently coloured compounds might be used for testing throughout this document. It is generally accepted that the colour is irrelevant for the properties discussed in this document.

3. Typical compound ingredients

3.1. Natural Rubber Latex

In line with current market expectations, compound FM480 does not contain any natural rubber or natural rubber latex.

3.2. Nitrosamines

Nitrosamines are residues of specific curing systems. These residues are in a number of cases known to be carcinogenic.
Rubber compound FM480 is formulated without making use of ingredients that potentially give rise to the formation of nitrosamines.

3.3. MCBT

2-mercaptobenzothiazole (2-MBT, 2-MCBT, MCBT) is a rubber chemical that is associated with a health risk.
Rubber compound FM480 does not contain 2-mercaptobenzothiazole (MCBT) or any of its derivatives.

3.4. BSE/TSE

Rubber compounds may contain components that are of animal origin. Most frequently it concerns fatty acids, fatty acid salts or esters that are either present as active components or as additives to active components.
Rubber compound FM480 does not use components of animal origin. FM480 is in full compliance with the European Pharmacopoeia 5.2.8., "Minimizing the risk of transmitting Animal Spongiform Encephalopathy Agents via medicinal products ".
(TSE = Transmissible Spongiform Encephalopathy; BSE = Bovine Spongiform Encephalopathy)

3.5. Heavy Metals

Both US and European legislation impose measures in order to prevent or reduce the impact of packaging and packaging waste on the environment.

Rubber compound FM480 fulfils the European Community Guideline 94/62/EC for heavy metals in packaging materials.
This directive states that packaging components shall not contain more than 100 ppm of Lead (Pb), Cadmium (Cd), Mercury (Hg) and Chromium (VI) (Cr).

EC Guideline 94/62/EC imposes the same requirements as the US CONEG regulation ('Toxics in Packaging Clearinghouse (TPCH)' as established by the Coalition of North-eastern Governors (CONEG) in 1992). FM480 thus also fulfills the CONEG requirements.

3.6. GMO (Genetically Modified Organisms)

Rubber compound FM480 does not contain ingredients that are derived from Genetically Modified Organisms (GMO).

3.7. Asbestos

Asbestos is known to pose a health hazard and has been subject of scrutiny by Health Authorities.
Datwyler does not use asbestos as ingredient in its rubber formulation. Also all possible precautions have been taken to have no asbestos fibers in production areas.

3.8. Phthalates

Phthalates are frequently used as plasticizer in plastics and are associated with health hazards.

Datwyler does not use this category of ingredients in its rubber formulations or in its processes. Compound FM480 is therefore free of phthalates and its derivatives.

3.9. Bisphenol A (BPA)

Bisphenol A (BPA) and BPA related substances may be present in certain plastic materials. They are associated with health hazards.

Datwyler confirms that FM480 does not use Bisphenol A or the following BPA related substances in its composition:

- Polycarbonate
- Polyether sulfone
- Polycarbonate/siloxane co-polymer
- Biostable polyurethanes
- Epoxy resin
- Bisphenol A diglycidylether methacrylate (BIS-GMA)
- Bisphenol A diglycidylether (BADGE)
- Bisphenol A dimethacrylate (BIS-DMA)
- Ethoxylated bisphenol A diacrylates.

4. Shelf Life

The shelf life of rubber compound FM480, intended for use in parenteral applications, stored in the original packaging under the ambient storage conditions as described in the ISO2230, “Rubber Products – Guideline for storage”, is 2 years after packing date.
In case a pre-treatment with gamma irradiation (25kGy) is applied, the recommended use is 1 year after packing date.

Hereafter, based on the indications given in the ISO2230, an additional shelf life of 5 years can be considered. Compatibility with the drug must be ascertained by the user.

5. Physical properties

5.1. Identification properties

The following tests are used to identify the rubber formulation following typical rubber technology standards.
The physical properties shown in Table 1 are taken for compound FM480/0, Gray. Properties like density and ash content may differ slightly for different colours of the said compound.
Data per compound colour are given on the corresponding Compound Data Sheets, available as separate documents upon request.

Table 1 : Physical properties – FM480/0

Hardness	°Shore A	ISO 7619 1 sec indentation	53 ± 5
Density	g/cm³	ISO 2781	1.317 ± 0.025
Ash	%	Internal Method(s): Calcination 4h@700°C	44.0 ± 2.0
Compression Set	%	ISO 815	max. 17
Tensile Strength	N/mm²	ISO 37	min. 4

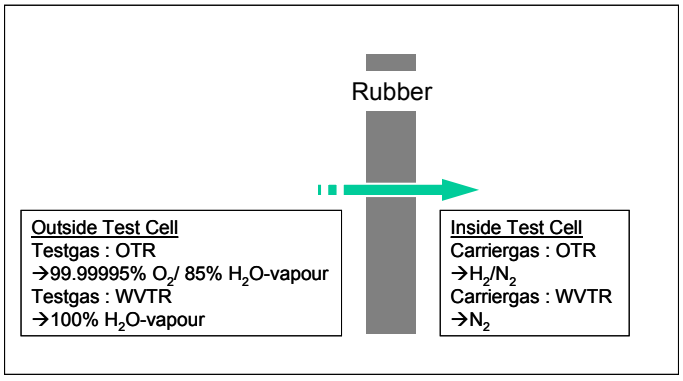
5.2. Permeability

Oxygen and water vapour transmission rates are measured as per ASTM D-3985 (oxygen) and ASTM F-1249 (water vapour). They measure the permeability of a material to oxygen viz. water vapour.
To be able to obtain permeability data on rubber compounds, a special mould, yielding a thin rubber slab, is used to prepare test pieces.
These rubber slabs, with a certain thickness (±1.3mm), are preconditioned at 23°C and 50% RH prior to the actual measurement.

For the test itself, a rubber slab is cut matching 50cm² and clamped in a double chamber test cell, acting as a barrier between both chambers (Figure 1).

The transmission rate is recorded once the system is at steady-state.

Figure 1 : Schematic principle of a transmission rate measurement



5.2.1. Water Vapour Transmission Rate (WVTR)

Equipment: **MOCON, Permatran-W 3/31 MG-module**
Conditions: **38 °C ; 100% relative humidity ; 100 flow N_2**

Table 2 : WVTR comparison

	WVTR in g/m².24h, 100% RH / 38°C
FM480	1.18
100% bromobutyl formulation	0.03
100% SBR formulation	3.90

5.2.2. Oxygen Transmission Rate (OTR)

Equipment: **MOCON, Oxtran 2/20 ML-module**
Conditions: **38 °C ; 85 % relative humidity ; 99.99995% O_2**

Table 3 : OTR comparison

	OTR in cc/m².24h, 85% RH / 38°C, 99.99995% O_2
FM480	207
100% bromobutyl formulation	48
100% SBR formulation	972

For reference purposes, the table also lists the permeability of a 100 % bromobutyl material and of a 100 % SBR rubber material.
FM480 ‘s permeability is intermediate between a typical bromobutyl and a typical SBR formulation. This is important in projects where ethylene oxyde (EtO) sterilization of assembled prefilled syringe components, notably tip caps, is considered. FM480 is enough permeable to EtO to exert its sterilizing function in the interface between the rubber tip cap and the prefillable syringe barrel. See in this respect section 11 of this Technical Documentation.

5.3. Influence of gamma irradiation on physical properties

FM480 displays unique characteristics in terms of permanent deformation upon gamma irradiation.
This paragraph lists the results of the impact of gamma sterilization on some physical properties of FM480. Measurements are performed at 2 irradiation levels: 30 kGy and 50 kGy. The former one is close to 25 kGy and represents a condition that is often met in practice. The dose of 50 kGy is chosen to illustrate the impact of worst-case conditions, due to uneven dose distribution or to a double sterilization at 25 kGy.
Results for a standard bromobutyl, often used for prefillable syringe plungers, are given for reference purposes.

Determination is done as per the relevant rubber standards and is carried out on the test objects described in those standards.

5.3.1. Hardness

Hardness is measured as per ISO 7619-1, ‘Rubber, vulcanized or thermoplastic -- Determination of indentation hardness -- Part 1: Durometer method (Shore hardness)’ .

Hardness is derived from the depth of indentation after a specific indentation time and under a specific force of an indenter into a rubber test piece and is translated in °Shore A.

Table 4 : Hardness before and after gamma irradiation

Compound	Unit	Before gamma	30 kGy	50 kGy
FM480	°Shore A	55.7	56.3	57.4
typical bromobutyl	°Shore A	50.6	52.2	52.1

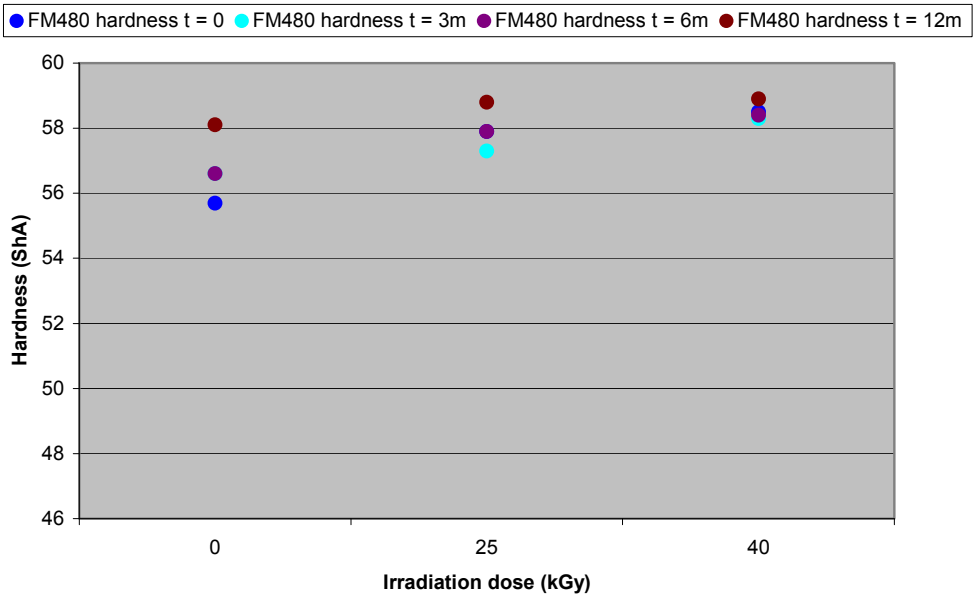
5.3.1.1. Hardness over time after gamma irradiation

The influence of gamma irradiation and subsequent storage on the hardness of the compound is evaluated.
The hardness of FM480 shows an increase with increasing irradiation dose. The increase is getting less important over time. Differences over time and after irradiation are rather small.

Table 5 : Hardness over time after gamma irradiation

Compound	Results (°Shore A) at different storage times				Irradiation dose
	T = 0m	T = 3m	T = 6m	T = 1 year	
FM480	55.7	56.6	56.6	58.1	**0 kGy**
	57.9	57.3	57.9	58.8	**25 kGy**
	58.5	58.3	58.4	58.9	**40 kGy**

Figure 2 : Hardness over time after gamma irradiation



5.3.2. Modulus

Modulus is measured as per ISO 37, 'Rubber, vulcanized or thermoplastic -- Determination of tensile stress-strain properties'**.**
Modulus is the force registered per unit area when a test piece is elongated to 100 or 300 % of its original length. In this respect one speaks of 'Modulus 100' and 'Modulus 300'.

Table 6 : Modulus 100 and 300 before and after gamma irradiation

Compound	Property	Unit	Before gamma	30 kGy	50 kGy
FM480	100 300	N/ mm²	2.18 2.98	2.47 3.13	2.51 3.73
typical bromobutyl	100 300	N/ mm²	2.08 3.71	2.16 3.32	2.23 3.00

5.3.3. Tensile strength / Elongation at break

These properties are equally measured as per ISO 37, 'Rubber, vulcanized or thermoplastic -- Determination of tensile stress-strain properties'**.**
Tensile strength ('TS' in the table below) is the maximum tensile stress recorded in extending the test piece to its point of breaking. Elongation at break ('EB' in the table below) is the elongation at this point.

Table 7 : Tensile strength / Elongation at break before and after gamma irradiation

Compound	Property	Unit	Before gamma	30 kGy	50 kGy
FM480	TS	N/ mm²	4.86	4.93	4.76
typical bromobutyl	TS	N/ mm²	8.86	6.35	3.99
FM480	EB	%	316	332	300
typical bromobutyl	EB	%	560	458	323

The properties of FM480 are relatively more insensitive to gamma irradiation than those of a typical bromobutyl compound. This holds especially for tensile strength and elongation at break.

5.3.4. Compression set

Compression set as per ISO 815-1 measures the ability of rubber to retain its elastic properties after prolonged compression at constant strain and at a specific set of conditions (24h, 25% compression, 70°C).

Table 8 : Compression set before and after gamma irradiation

Compound	Unit	Before gamma	30 kGy	50 kGy
FM480	%	14.6	14.1	14.0
typical bromobutyl	%	15.2	16.9	19.5

The impact of gamma irradiation on compression set is noted to be quasi inexistent for FM480.

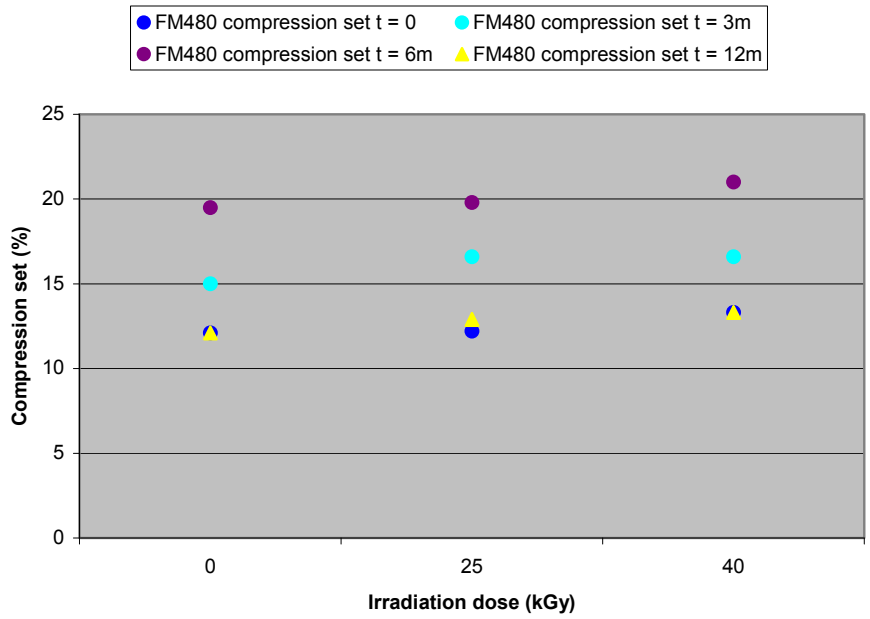
5.3.4.1. Compression set over time after gamma irradiation

Gamma irradiation has little to no effect on the compression set of FM480.
Further storage over time causes a slight increase of the compression set during the first 6 months, also for not irradiated test pieces. At 1 year, compression set is in line with the data at time point '0 months'.

Table 9 : Compression set over time after gamma irradiation

Compound	Results (%) at different storage times				Remarks
	T = 0m	T = 3m	T = 6m	T = 1 year	
FM480	12.1	15.0	19.5	12.1	0 kGy
	12.2	16.6	19.8	12.9	25 kGy
	13.3	16.6	21.0	13.3	40 kGy

Figure 3 : Compression set over time and after gamma irradiation



5.3.5. Irradiation set

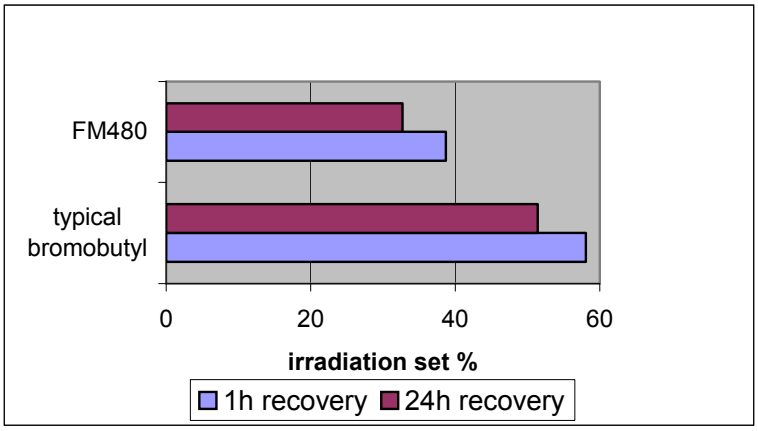
Every rubber material is characterized by a permanent deformation, i.e. after imposing a mechanical deformation for a specified time and then taking away the source of deformation, rubber will not 100 % recover its original form. The permanent deformation left is termed 'compression set'. The standard for measuring compression set is ISO 815-1, 'Rubber, vulcanized or thermoplastic -- Determination of compression set -- Part 1: At ambient or elevated temperatures'. See also above.

The permanent deformation of rubber is known to be higher if at the same time as compression an irradiation takes place. In this case, one may speak of 'irradiation set'. Irradiation set is measured by an internal test method where the rubber test parts are irradiated under strong deformation conditions similar to ISO 815-1 (25 % reduction of original height of cylindrical test buttons).
The time between the start of compression/gamma irradiation and the final irradiation set measurement is fixed at 2 weeks.

Table 10 : Irradiation set

Compound	Irradiation set 25 kGy 1 h after release of deformation	Irradiation set 25 kGy 24 hrs after release of deformation
FM480	38.7%	32.7%
typical bromobutyl	58.1%	51.4%

Figure 4 : Irradiation set



A lower irradiation set provides a better assurance of non-deformation of rubber parts that are irradiated in compressed condition. The latter is typical for RTP (Rapid Transfer Port) applications.

It also prevents deformation of plungers that are irradiated in assembled condition in prefillable syringe barrels, as may be the case with plastic prefillable syringes.

5.3.6. Sterilizability

FM480 can be sterilized by steam or by gamma irradiation.

An investigation specifically on EtO sterilization has been performed. Testing and conclusions are summarized in chapter 11 of this document.

6. Chemical properties before and after gamma irradiation

6.1. Pharmacopeial data

6.1.1. Pharm. Eur. 3.2.9 / USP <381> data

A revised version of USP <381>, has come into force on May 1, 2009. Sample preparation, test description and the 2-tier acceptance criteria were largely harmonized with the Pharm. Eur. 3.2.9.

The table below summarizes the results of FM480 chemical testing according to both USP <381> and Pharm. Eur. 3.2.9.

Table 11 : Pharm. Eur. 3.2.9 / USP <381> data, chemical part

Characteristic		Amount tested	Units	Limit	Typical Value	
Appearance of solution	Turbidity	Sol. S	NTU	Type I: 6.0 *(*)* Type II: 18.0 *(*)*		0.2
	Colour	Sol. S		See test procedure		pass
Acidity or alkalinity		Sol. S (20 ml)	ml 0.01M HCl ml 0.01M NaOH	0.8 0.3	Blank / 0.07 EP USP	/ 0.07 0.07 0.00
Absorbance		Sol. S	A_{max}220-360 nm	Type I: 0.2 Type II: 4.0		0.02
Reducing substances		Sol. S (20 ml)	ml 0.002M $KMnO_4$	Type I: 3.0 Type II: 7.0		0.21
Extractable heavy metals		Sol. S	ppm Pb^{2+}	2	EP USP	<2 <2
Extractable zinc		Sol. S	ppm Zn^{2+}	5.0		0.01
Ammonium		Sol. S	ppm NH_4^+	2		<2
Residue on evaporation (only for EP)		Sol. S (50 ml)	mg	Type I: 2.0 Type II: 4.0		0.5
Volatile sulphides		20 cm²	mg S^{2-}	0.02		<0.02

* *By definition corresponding with reference suspensions II and III respectively.*

6.1.2. Pharm. Eur. 3.2.9 over time after gamma irradiation

The following table lists the chemical properties of FM480 when measured according to Pharm. Eur. 3.2.9. “Rubber closures for containers for aqueous parenteral preparations, for powders and for freeze-dried powders”, valid version.

Following the gamma irradiation, uncut rubber closures with a total surface of 100cm² are boiled for 5 min in distilled water and rinsed 5 times with cold distilled water. Next, a rubber/water ratio of 100cm² rubber/200 ml distilled water is autoclaved for 30min at 121°C (Solution S).

Pharm. Eur. 3.2.9 results are followed up as a function of time after irradiation and this for various irradiation doses up to 40 kGy.

Table 12 : Pharm.Eur.3.2.9. Chemical properties over time after gamma irradiation

Characteristic	Amount tested	Units	Limit	FM480											
				0kGy				25kGy				40kGy			
				T = 0m	T = 3m	T = 6m	T=1yr	T = 0m	T = 3m	T = 6m	T=1yr	T = 0m	T = 3m	T = 6m	T=1 yr
Appearance of solution – turbidity	Solution S	NTU	Type I: 6.0	0.17	0.13	0.37	0.42	0.11	0.11	0.39	0.54	0.14	0.14	0.42	0.53
			Type II: 18.0	-	-	-	-	-	-	-	-	-	-	-	-
Appearance of solution – colour	Solution S		Pass/fail	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
Acidity/alkalinity	20ml Solution S	ml 0.01M HCl or	0.8 ml 0.01M HCl	-	-	-	-	-	-	-	-	-	-	-	-
		ml 0.01M NaOH	0.3 ml 0.01M NaOH	0.08	0.06	0.05	0.06	0.08	0.06	0.05	0.06	0.08	0.06	0.05	0.06
Absorbance	Solution S	Amax 220-360 nm	Type I: 0.2	0.02	0.02	0.02	0.01	0.03	0.02	0.03	0.02	0.03	0.03	0.04	0.02
			Type II: 4.0	-	-	-	-	-	-	-	-	-	-	-	-
Reducing substances	20 ml Solution S	ml 0.002M KMnO4	Type I: 3.0	0.15	0.12	0.16	0.12	0.25	0.22	0.39	0.23	0.2	0.11	0.27	0
			Type II: 7.0	-	-	-	-	-	-	-	-	-	-	-	-
Extractable heavy metals	Solution S	ppm Pb2+	2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
Extractable Zinc	Solution S	Ppm Zn2+	5	<0.01	0	<0.01	0.06	<0.01	0	<0.01	0.05	<0.01	0	<0.01	0.07
Ammonium	Solution S	Ppm NH4+	2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
Residue on evaporation	50ml Solution S	Mg	Type I: 2.0	0.1	0.2	0.4	0.1	0.1	0.4	0.10	0	0	0.3	0.5	0
			Type II: 4.0	-	-	-	-	-	-	-	-	-	-	-	-
Volatile sulphides	20 cm²	mg S2-	0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Conductivity*	Solution S	µS/cm	Type I: 15	1.04	0.93	0.35	0.40	0.93	1.09	0.55	0.35	0.57	0.93	0.39	0.36
			Type II: 30	-	-	-	-	-	-	-	-	-	-	-	-

* Conductivity is an optional test to the Pharm. Eur. 3.2.9. described in the ISO8871-1

All results are largely within Type I limits of Pharm. Eur. 3.2.9.
There is little to no influence of gamma irradiation on the chemical properties of FM480 when tested according Pharm. Eur. 3.2.9., also not after further storage after irradiation.
In view of the above-mentioned harmonization of USP <381> and Pharm. Eur. 3.2.9 chemical testing, this conclusion equally holds for USP <381>.

6.1.3. Japanese Pharmacopeia 7.03

Results for FM480, tested according to physicochemical part of the Japanese Pharmacopeia, 14th edition, Part I, chapter <59> (identical to Pharm. Jap. chapter 7.03, current edition), "Test for Rubber Closures for Aqueous Infusions", are given in Table 13 below. 30 g of rubber sample is autoclaved in 300 g distilled water for 60 min. at 121°).

Due to the peculiar definition of the sample preparation (by rubber mass and not by rubber surface), results are closure design dependent (surface/volume ratio dependency). Results in the table are given for a syringe plunger that has a surface/volume ratio comparable to a 20mm injection closure as described in ISO 8362-2.

For the design given, FM480 complies with the extractable substances part of the Pharm. Jap. 7.03. Other parts of the Pharm. Jap. 7.03 were not tested and hence are not documented. Please contact your Datwyler sales representative in case of need.

Table 13 : JP 7.03

CRITERIUM	AMOUNT TESTED	UNITS	LIMITS	RESULTS		
				0kGY	25kGy	50kGy
Appearance (430-650 nm)	10 mm cuvet	%T at 430 nm %T at 650 nm	99% T 99% T	99.47 99.51	99.86 99.89	99.75 99.78
Foam test	5 ml	-	foam disap. < 3 min.	pass	pass	pass
pH	20 ml	pH units	difference with blank: max. 1.0	0.31	0.27	0.21
Reducing substances	100 ml	ml 0.002 M $KMnO_4$	2.0	0.72	1.36	1.26
Evaporation residue	100 ml	mg	2.0	0.33	0.70	0.40
UV absorb. (220-350 nm)	10 mm cuvet	absorbance	0.2	0.028	0.048	0.048
Zinc	10 ml	ppm Zn^{2+}	1 ppm	0.003	0.005	0.042

There is little to no influence of gamma irradiation on the chemical properties of FM480 when tested according the Pharm. Jap. 7.03.

6.2. ISO 8871-1

The requirements of the ISO8871-1, "Elastomeric parts for parenterals and for devices for pharmaceutical use – Part 1: Extractables in aqueous autoclavates", are identical to those set down in Pharm. Eur. 3.2.9. that in turn since May 1, 2009 is largely harmonized with USP <381>.

6.3. Extractable study

A detailed extractables study on FM480 involving solvents of various polarity is available on request.

Because of their confidential nature, the results of this extractables study are not given in full detail in this report. They will be available only after conclusion of specific agreements. Please contact your Datwyler Sales representative.

7. Testing in contact with WFI

WFI (Water-for-Injection) because of its purity is a very powerful extractant in contact with elastomeric materials. Under these conditions the WFI that has been prepared by suitable purification steps starting from potable water will try to saturate itself in organic and inorganic compounds that are available for extraction in the elastomeric material.

In this respect WFI is a very thankful medium to compare the extractable properties of different rubber materials.

In this section the WFI extractable properties of FM480 will be compared with those of a typical bromobutyl compound. A number of pertinent properties that various pharmacopoeia impose on packaged Water-for-Injection are investigated.
They are complemented by a number of tests like determination of zinc extraction and determination of a UV spectrum of an aqueous (WFI) extract that are typical for rubber testing.
Following test criteria will be discussed in this section:

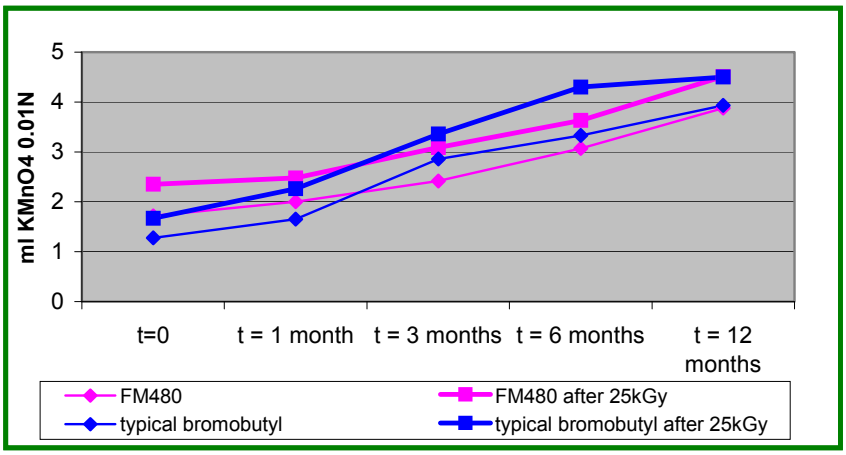
- Reducing substances (Pharm.Jap.);
- Chlorides (Pharm Eur);
- Chlorides/Bromides (Quantitative analyses via IC);
- Zn, Ca, Mg, (Quantitative analyses via ICP);
- pH (Pharm. Jap.);
- Acidity/Alkalinity (Pharm. Eur.);
- UV absorption (Pharm.Eur).

At time = 0 the elastomeric material is sterilized in WFI for 30 minutes at 121 °C in a ratio of 2.34 cm² of rubber surface area per 1 ml of WFI. After that time storage takes place at 40 °C with the rubber still in contact with the WFI.
The ratio of 2.34 cm² of rubber per 1 ml of WFI is extremely unfavourable. For reference, the pharmacopoeia extraction ratio for rubber is 1 cm² of rubber per 2 ml of water (USP <381> and Pharm. Eur. 3.2.9). Equally the condition of 2.34 cm² of rubber per 1 ml of WFI would correspond with a 0.5 ml syringe filled with 0.1 ml of water.

Tests are carried out on non-irradiated and on irradiated (25 kGy) closures. This allows verifying the impact of irradiation on extractable levels in water.

7.1.1. Reducing substances

Figure 5 : Reducing substances before and after 25kGy gamma irradiation

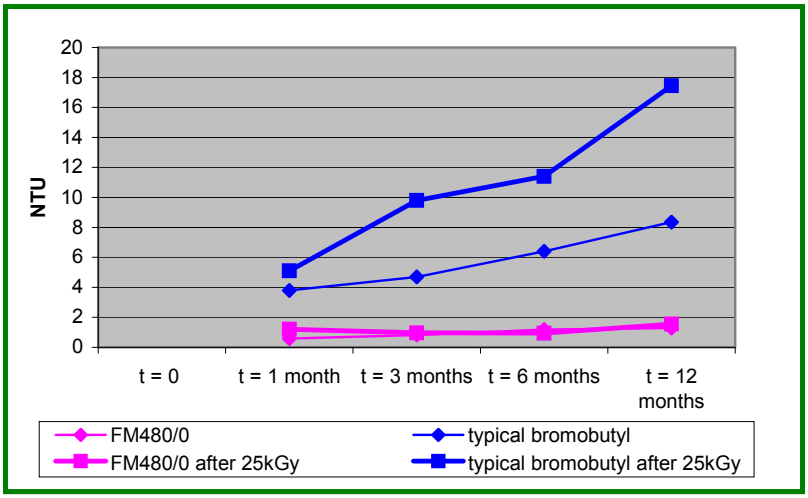


For 'reducing substances', the behaviour of both formulations is very similar. Irradiation has a modest influence on the results.

7.1.2. Chlorides/Bromides (NTU)

Chloride and bromide ions are stemming from the halobutyl polymer. The presence of chloride and bromide is measured via a precipitation reaction with silver nitrate and is quantified by measuring the turbidity of the precipitate solution. Turbidity is expressed as Nephelometric Turbidity Units (NTU).
The NTU test method does not allow distinguishing between the 2 types of ions. Therefore additionally the bromide and the chloride ion were quantified separately by measuring with Ion Chromatography. These results are given in the next paragraph.

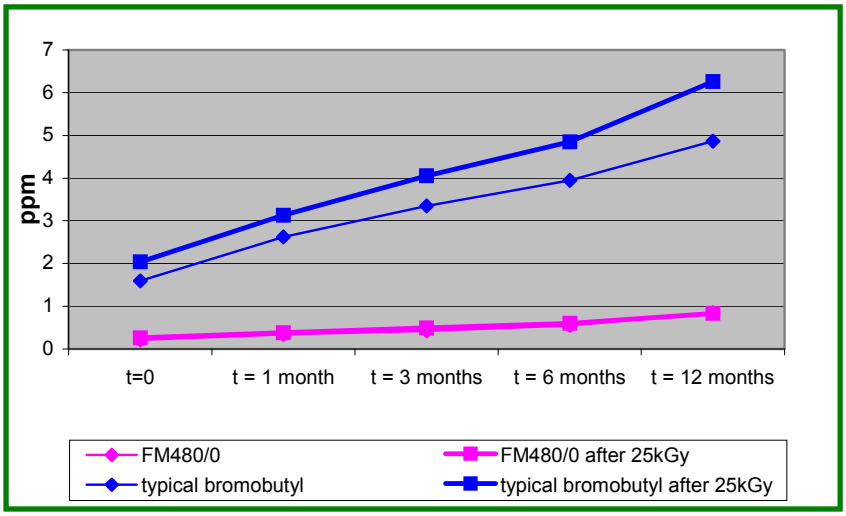
Figure 6 : Chlorides/Bromides before and after 25kGy gamma irradiation



The release of chloride/bromide with FM480 is clearly less than the typical bromobutyl. For FM480, there is hardly any evolution in release of Cl^-/Br^- over time and the impact of irradiation is negligible.

7.1.3. Bromides via Ion Chromatography

Figure 7 : Bromides via IC before and after 25kGy gamma irradiation

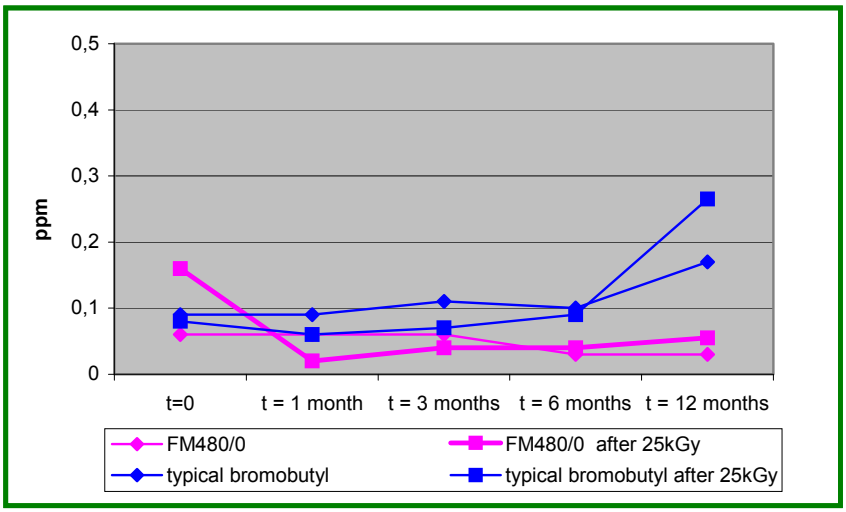


** note: different scaling in y-axis is used in Figure 7 and Figure 8.*

The results of the previous paragraph are confirmed here, i.e. the release of bromide ion with FM480 is clearly less than with the typical bromobutyl. For FM480 over time, there is hardly any evolution in release of Br^- and the impact of irradiation is practically inexistent.

7.1.4. Chlorides via Ion Chromatography

Figure 8 : Chlorides via IC before and after 25kGy gamma irradiation



** note: different scaling in y-axis is used in Figure 7 and Figure 8.*

Clearly much less chloride than bromide ions are extracted. This is explained by the fact that both compounds under study are bromobutyl based compounds.

7.1.5. Zinc, Calcium and Magnesium

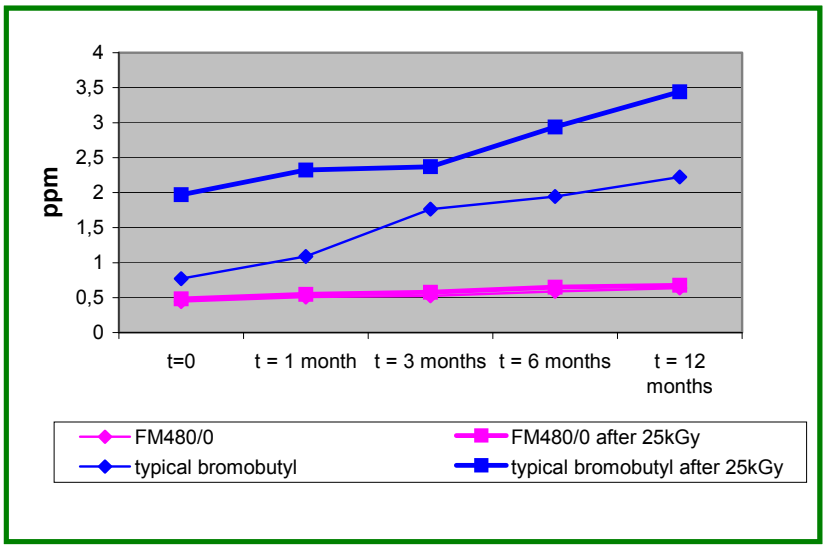
Zinc has been a commonly used chemical as part of many rubber formulations and is easily extracted in aqueous solutions. Though Zn ions are typically undesired in certain drug products.

Calcium and magnesium are 2 ions for which there are pharmacopeial limits on Water-for-Injection. The pharmacopeia prescribes measurement of the sum of Ca and Mg by wet chemistry (titration with EDTA).

For this study Zn, Ca and Mg ions are determined via AAS (Atomic Absorption Spectrophotometry).

The sum of the cations Zn, Ca and Mg ions is given.

Figure 9 : Zn, Ca and Mg ion extractables before and after 25kGy

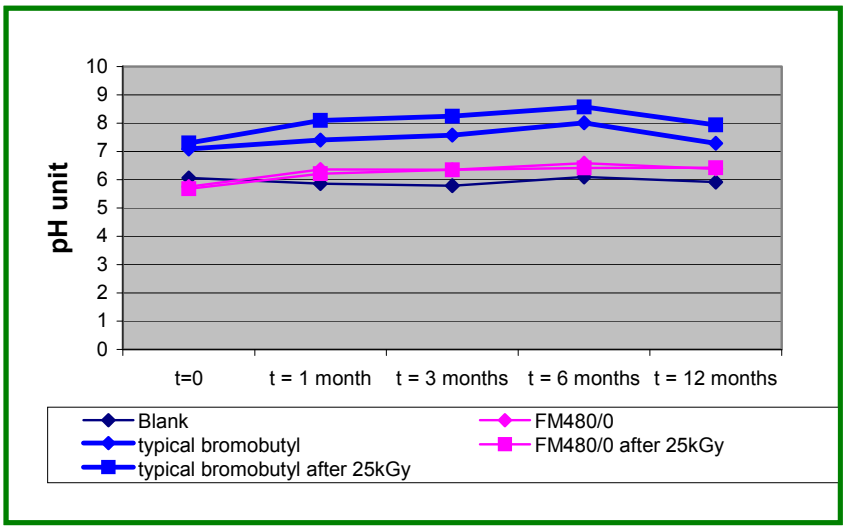


Zn ions from FM480 as well as from the typical bromobutyl under study, both from irradiated and from non-irradiated components, are measured at ppb level, i.e. at trace levels only. FM 480 therefore can be considered Zn free.

The total cation extractable level for FM480 is very low and is not affected by irradiation. The behaviour of FM480 comes very close to the behaviour of a compound dedicated for contact with WFI.

7.1.6. pH (Alkalinity/acidity)

Figure 10 : pH before and after 25kGy gamma irradiation



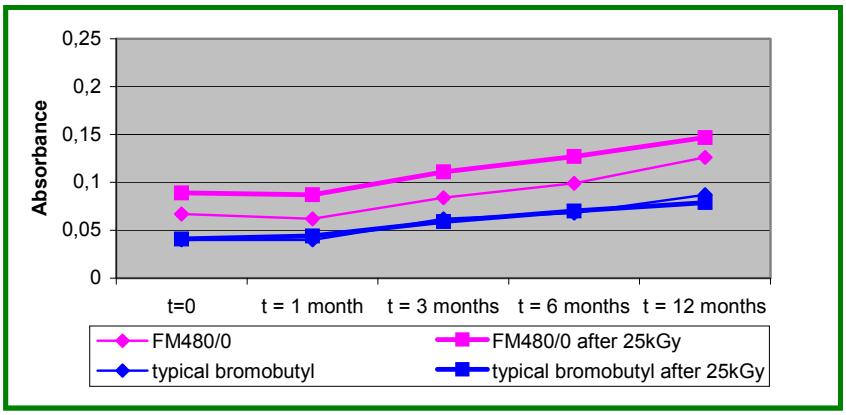
FM480 is almost pH neutral, i.e. the originally installed pH, also under the most unfavourable conditions of the test is kept constant over time.

Gamma irradiation does not affect this pH stability.

7.1.7. UV absorbance

UV absorbance is indicative for the organic extractable level of a rubber material. Measuring UV absorbance makes part of the pharmacopeial requirements for assessing aqueous extractables from elastomeric materials.
UV absorbance is measured in the range of 190 to 360 nm. The maximum absorbance noticed in this range is taken as the result.

Figure 11 : UV absorbance before and after 25kGy gamma irradiation



UV absorbance for FM480 is found to be low. A slight increase can be seen both over time and after a gamma irradiation with 25kGy.

8. Functional Properties

FM480 is intended to serve as compound material for elastomeric closures for prefillable syringes (plungers, tip caps) and not as material for vial stoppers. Therefore, the focus for functional properties lays in the gliding behavior of plunger stoppers rather than the functional properties like penetrability, fragmentation and resealability as described in the Pharm. Eur. 3.2.9.

This section of the Technical Documentation describes the gliding behavior of FM480 plungers for prefillable syringes, while section 11 discusses the ethylene oxide sterilization properties of tip caps in FM480.

In general, the gliding behavior of plungers is analyzed as activation force on one hand and gliding force on the other hand:

- activation force is the force needed to bring the plunger in motion;
- gliding force is defined as the force needed to keep the plunger moving after the activation.

For this study, glass barrels of size 1ml with silicone oil, with staked needle, available from the market were used.
The plungers were siliconized ISAF2, the standard Datwyler siliconisation level for this type of product. The 1-3ml plunger design used can be found in attachment 13.1 on page 38.
The siliconization level was measured in duplicate with an AAS-method. The amount of silicone on rubber closures is evaluated by extracting a number of stoppers in methyl isobutyl ketone (MIBK), followed by determination of Si (silicon) in the resulting extract via AAS.

Table 14 : AAS results for ISAF2 treated plungers

Plunger sample	µg silicone oil / cm²
FM480/0 V9258 SAF2 CH0742904	32 32 AVG = 32 µg/cm²

Prior to assembly in the syringe barrels, the plungers were irradiated at a level of 25 kGy.
Forces were measured at a plunger speed of 100mm/min using a Zwick tensile bench.

8.1. Gliding behavior in a typical glass syringe

Glass barrels were filled with WFI and then steam sterilized for 30min at 121°C.
Barrels are stored in a ventilated oven at 40°C or at room temperature.
The 40 °C study is a three months study; the room temperature study is a 12 months study.

8.1.1. Gliding behavior of non-irradiated plungers in a glass syringe

The activation force is defined as the highest peak registered at the start of the gliding curve. The gliding force is defined as the maximum force value registered during the further path length in the gliding curve. Average values are shown in the below table.

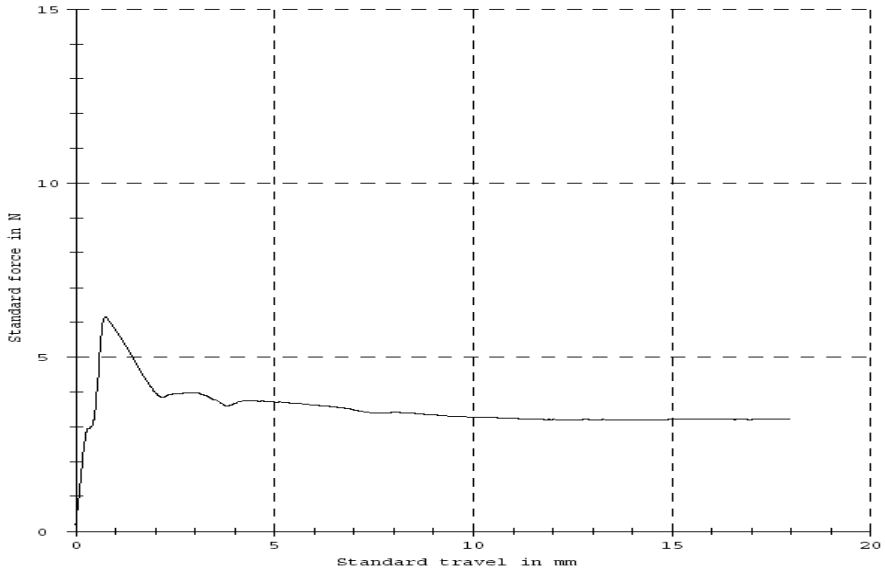
Figure 12 : Activation and gliding forces for a 1-3ml plunger design – study at room temperature

Compound	Product		T_1 = 1 month RT	T_3 = 3 months RT	T_6 = 6 months RT	T_{12}= 12months RT
FM480/0	1-3ml plunger (V9258)	Activation	5.3	5.9	6.4	7.4
		Gliding	4.0	4.0	4.0	3.5
typical bromobutyl	1-3ml plunger (V9258)	Activation	9.2	11.4	12.6	14.4
		Gliding	12.0	16.0	14.0	14.5

Table 15 : Activation and gliding forces for a 1-3ml plunger design – accelerated study at 40°C

Compound	Product		T_0= 1 week 40°C	T_1 = 1 month 40°C	T_3 = 3 months 40°C
FM480/0	V9258	Activation	4.6	5.9	6.2
		Gliding	4.0	4.0	3.5
typical bromobutyl	V9258	Activation	10.5	13.4	15.3
		Gliding	12.0	15.0	13.0

Figure 13 : Typical gliding curves for a 1-3ml plunger design in FM480 – 3 months/40°C



8.1.2. Activation forces of irradiated plungers (as function of storage time after gamma irradiation)

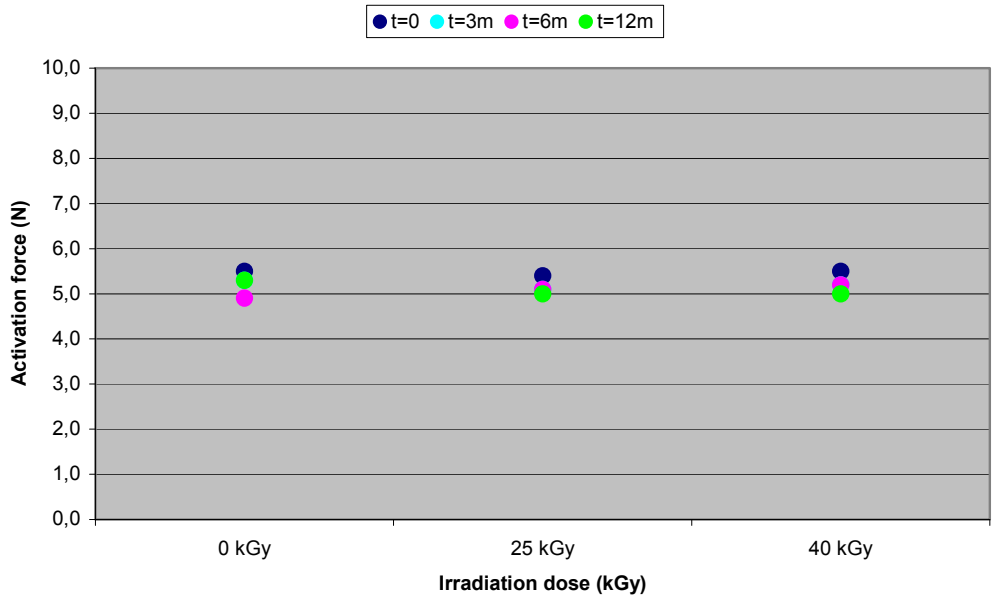
This paragraph illustrates the activation forces for ISAF2 treated 1-3 ml plungers V9258 as a time function after irradiation with various gamma doses.

Plungers at various time points up to 1 year after irradiation were assembled in glass barrels. The barrels were autoclaved at 121 °C during 30 minutes in the lab autoclave.
After autoclavation the barrels were further stored at 40 °C for 1 week. Hereafter they were used for evaluation of activation force.

Table 16 : Activation forces over time after gamma irradiation – FM480

Product	Storage Time	Activation force (N)		
		0 kGy	25 kGy	40 kGy
V9258 FM480 ISAF2	T = 0 m	5.5	5.4	5.5
	T = 3 m	5.3	5.1	5.2
	T = 6 m	4.9	5.1	5.2
	T = 1 yr	5.3	5.0	5.0

Figure 14 : Activation forces over time after gamma irradiation – FM480



No noticeable difference was observed in activation force for the different irradiation levels at any of the evaluation points up to 1 year storage of the plungers.

8.2. Plunger/barrel seal integrity (PBSI): dye ingress method

8.2.1. PBSI behavior of non-irradiated plungers in a glass syringe

20 water filled, steam sterilized syringes are stored at 40°C during 1 week.
These water filled barrels are submerged in a methylene blue solution that is placed under an atmosphere at 300mbar below ambient atmospheric pressure.
After 16 hours, the pressure over the immersed syringes is brought back to atmospheric pressure and the dye bath with the syringes is left to stand for 30 minutes.
After 30 minutes, the syringes are rinsed with water and inspected for leakage past the seals.
No leakage of methylene blue, past the seals of the plunger, is allowed.

Table 17 : PBSI for 1-3ml design plunger: dye ingress method (1week@40°C)

Compound	Product	T_0= 1 week 40°C
FM480/0	V9258	0/20
typical bromobutyl	V9258	0/20

All syringes (20 syringes per plunger sample) successfully pass the dye test method after storage of the syringes during 1 week at 40°C.

8.2.2. PBSI behavior of irradiated plungers (as function of storage time after gamma irradiation)

The same experiment as in the previous section was repeated, this time however with FM480 plungers that had been irradiated at different gamma doses and that had been stored up to 1 year after irradiation.

Table 18 : PBSI over time after gamma irradiation – FM480

Product	Storage Time	Number of leakage/tested barrels		
		0 kGy	25 kGy	40 kGy
V9258 FM480 ISAF2	T = 0 m	0/20	0/20	0/20
	T = 3m	0/20	0/20	0/20
	T = 6m	0/20	0/20	0/20
	T = 1 yr	0/20	0/20	0/20

No leaks were observed at any of the evaluation points and at any irradiation dose under study.

9. Biological Properties

9.1. USP <1031>

The USP<1031>, “The Biocompatibility of Material used in Drug Containers”, stipulates that the biocompatibility of an elastomeric material is evaluated according to the two stage testing protocol specified in the USP<381>. An elastomeric material that does not meet the requirements of the first-stage testing (in vitro, USP<87>), may qualify as a biocompatible material by passing the second stage testing (in vivo, USP<88>).

No class or type distinction is made between elastomeric materials that meet the requirements of first-stage of testing and those that qualify as biocompatible meeting the second-stage requirements.

9.2. USP <87>

9.2.1. USP <87> behavior of non-irradiated FM480

Biological testing (-elution test-) is carried out on a sample of FM480 as per the USP<87>, “Biological Reactivity Tests, In Vitro”.
FM480 proves to be non-cytotoxic, a copy of the report can be found in Figure 15 .

9.2.2. USP <87> behavior of irradiated FM480 (as function of storage time after gamma irradiation)

The same elution test as per USP<87> is performed on products after 12 months storage after gamma irradiation at various gamma doses up to 40 kGy.
FM480 remains non-cytotoxic under the given circumstances, as is shown in the test result certificates on the next pages (Figure 16, Figure 17, Figure 18).

9.3. ISO 8871-4

The ISO 8871-4, “Elastomeric parts for parenterals and for devices for pharmaceutical use – Part 4: Biological requirements and test methods”, specifies biological requirements for bacterial endotoxins, bioburden, cytotoxicity and intracutaneous and systemic toxicity.

The requirements for endotoxins and bioburden are left open and shall be agreed upon between supplier and user.

For the toxicity tests, the same approach as in the USP<1031> is given, including reference to the USP<87>, in vitro test, for the cytotoxicity test and the USP<88>, in vivo test for the intracutaneous and systemic toxicity test.

Figure 15 : Elution test (USP<87>), 0kGy – time point = 0 months
(note: H5-30-47/0 is the development code name for FM480/0)

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DATWYLER

TEST RESULT CERTIFICATE

Project Number:	**TE 03486**	**Study Number:**	**03-B1408-N1**
Sponsor:	**Helvoet Pharma**	**Report Date:**	**21/11/2003**
Contact:	*Mr. L. Vanderheyden*		
Address:	Industriepark Kolmen B-3570 Alken	Technical Initiation: Technical Completion:	17/11/2003 20/11/2003
PO.Number:	001235		

Study	**Elution Test – USP**	Temp/Time	37°C/24 hours
Test Item	H5-30-47/0	Ratio	60 cm²/ 20 ml
Lot	320905	Vehicle	MEM-Complete

REFERENCE: Based on USP 26-NF 21, 2003: <87> Biological reactivity test, in vitro.
Toxikon Reference: SOP 3.1.2.3, rev.02.

PROCEDURE: The biological reactivity of a mammalian monolayer, L929 mouse fibroblast cell culture, in response to the test item extract was determined. Extracts were prepared at 37±1°C for 24 hours in a humidified atmosphere containing 5±1% carbon dioxide. Positive (natural rubber) and negative (silicone) control articles were prepared to verify the proper functioning of the test system. The maintenance medium on the cell cultures is replaced by the extracts of the test item or control article in duplicate and the cultures are subsequently incubated for 48 hours, at 37±1°C, in a humidified atmosphere containing 5±1% carbon dioxide. Biological reactivity was rated on a scale from Grade 0 (No reactivity) to Grade 4 (Severe reactivity).
The test item meets the requirements of the test if none of the cultures exposed to the test item shows greater than mild reactivity (Grade 2).

RESULTS: No reactivity (Grade 0) was exhibited by the cell cultures exposed to the test item at the 48 hours observation. Severe reactivity (Grade 4) was observed for the positive control article. The negative control article showed no signs of reactivity (Grade 0).

CONCLUSION: Based on the evaluation criteria mentioned above, the test item is considered non-cytotoxic.

RECORD STORAGE: All raw data generated in this study will be archived at Toxikon Europe, according to SOP 4.2.8.

AUTHORIZED PERSONNEL

Dr. Ine Janssen
Study Director

Ing. Ingrid Lenotte
Quality Assurance

This copy will not be systematically updated !

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Fortis Bank 230-0391575-06 - KBC Bank 431-0597001-33 - BTW/TVA BE 442.395.719 - H.R. Leuven 80.154

Figure 16 : Elution test (USP<87>), 0kGy – time point = 12 months

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TEST RESULT REPORT

Project Number:	**TE 09358**	**Study Number:**	**09-B0823-N1**
Sponsor:	**Helvoet Pharma Belgium NV**	**Report Date:**	**15/05/2009**
Contact:	*Mrs. Nadia Nouri*		
Address:	Industrieterrein Kolmen 1519 B-3570 Alken, Belgium	Date Sample Arrival:	12/05/2009
		Technical Initiation:	12/05/2009
PO.Number:	PB0901530	Technical Completion:	15/05/2009

Study	**Elution Test - ISO**	Temp/Time	37°C/24 hours
Test Item	V9258 FM480/0 0kGY Gamma t=12m	Ratio	25 cm²/20 mL
Lot	Ch808933	Vehicle	MEM-Complete

REFERENCE: According to "ISO 10993-5, 1999: Biological Evaluation of Medical Devices- Part 5:Tests for In Vitro Cytotoxicity." and "USP 32-NF 27, 2009: <87> Biological reactivity test, in vitro." Toxikon Reference: SOP 3.1.2.3, rev. 06

PROCEDURE: The biological reactivity of a mammalian monolayer, L929 mouse fibroblast cell culture, in response to the test item extract was determined. Extracts were prepared at 37±1°C for 24 hours in a humidified atmosphere containing 5±1% carbon dioxide (static). Positive (natural rubber) and negative (silicone) control articles were prepared to verify the proper functioning of the test system. The pH of the extracts was measured and the extracts sterile filtered. The maintenance medium on the cell cultures is replaced by the extracts of the test item or control article in triplicate and the cultures are subsequently incubated for 48 hours, at 37±1°C, in a humidified atmosphere containing 5±1% carbon dioxide. Biological reactivity was rated on the following scale: Grade 0 (No reactivity); Grade 1 (Slight reactivity), Grade 2 (Mild reactivity), Grade 3 (Moderate reactivity) and Grade 4 (Severe reactivity). The test item is considered non-cytotoxic if none of the cultures exposed to the test item shows greater than mild reactivity (Grade 2).

RESULTS: No reactivity (Grade 0) was exhibited by the cell cultures exposed to the test item at the 48 hours observation. Severe reactivity (Grade 4) was observed for the positive control article. The negative control article showed no signs of reactivity (Grade 0).

CONCLUSION: Based on the evaluation criteria mentioned above, the test item is considered non-cytotoxic.

RECORD STORAGE: All raw data generated in this study will be archived at Toxikon Europe, according to SOP 4.2.8.

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Fortis 230-0391575-06 - KBC 431-0597001-33 - BTW/TVA BE 0442.395.719 - H.R. Leuven 80.154

Figure 17 : Elution test (USP<87>), 25kGy – time point = 12 months

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TEST RESULT REPORT

Project Number:	**TE 09358**	**Study Number:**	**09-B0817-N1**
Sponsor:	**Helvoet Pharma Belgium NV**	**Report Date:**	**15/05/2009**
Contact:	*Mrs. Nadia Nouri*		
Address:	Industrieterrein Kolmen 1519 B-3570 Alken, Belgium	Date Sample Arrival: Technical Initiation:	12/05/2009 12/05/2009
PO.Number:	PB0901530	Technical Completion:	15/05/2009

Study	**Elution Test - ISO**	Temp/Time	37°C/24 hours
Test Item	V9258 FM480 0 25kGy Gamma t=12m	Ratio	25 cm²/20 mL
Lot	Ch808933	Vehicle	MEM-Complete

REFERENCE: According to "ISO 10993-5, 1999: Biological Evaluation of Medical Devices- Part 5:Tests for In Vitro Cytotoxicity." and "USP 32-NF 27, 2009: <87> Biological reactivity test, in vitro." Toxikon Reference: SOP 3.1.2.3, rev. 06

PROCEDURE: The biological reactivity of a mammalian monolayer, L929 mouse fibroblast cell culture, in response to the test item extract was determined. Extracts were prepared at 37±1°C for 24 hours in a humidified atmosphere containing 5±1% carbon dioxide (static). Positive (natural rubber) and negative (silicone) control articles were prepared to verify the proper functioning of the test system. The pH of the extracts was measured and the extracts sterile filtered. The maintenance medium on the cell cultures is replaced by the extracts of the test item or control article in triplicate and the cultures are subsequently incubated for 48 hours, at 37±1°C, in a humidified atmosphere containing 5±1% carbon dioxide. Biological reactivity was rated on the following scale: Grade 0 (No reactivity); Grade 1 (Slight reactivity), Grade 2 (Mild reactivity), Grade 3 (Moderate reactivity) and Grade 4 (Severe reactivity). The test item is considered non-cytotoxic if none of the cultures exposed to the test item shows greater than mild reactivity (Grade 2).

RESULTS: No reactivity (Grade 0) was exhibited by the cell cultures exposed to the test item at the 48 hours observation. Severe reactivity (Grade 4) was observed for the positive control article. The negative control article showed no signs of reactivity (Grade 0).

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Figure 18 : Elution test (USP<87>), 40kGy – time point = 12 months

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TEST RESULT REPORT

Project Number:	**TE 09358**	**Study Number:**	**09-B0820-N1**
Sponsor:	**Helvoet Pharma Belgium NV**	**Report Date:**	**15/05/2009**
Contact:	*Mrs. Nadia Nouri*		
Address:	Industrieterrein Kolmen 1519 B-3570 Alken, Belgium	Date Sample Arrival: Technical Initiation:	12/05/2009 12/05/2009
PO.Number:	PB0901530	Technical Completion:	15/05/2009

Study	**Elution Test - ISO**	Temp/Time	37°C/24 hours
Test Item	V9258 FM480/0 40kGy Gamma t=12m	Ratio	25 cm²/20 mL
Lot	Ch808933	Vehicle	MEM-Complete

REFERENCE: According to "ISO 10993-5, 1999: Biological Evaluation of Medical Devices- Part 5:Tests for In Vitro Cytotoxicity." and "USP 32-NF 27, 2009: <87> Biological reactivity test, in vitro." Toxikon Reference: SOP 3.1.2.3, rev. 06

PROCEDURE: The biological reactivity of a mammalian monolayer, L929 mouse fibroblast cell culture, in response to the test item extract was determined. Extracts were prepared at 37±1°C for 24 hours in a humidified atmosphere containing 5±1% carbon dioxide (static). Positive (natural rubber) and negative (silicone) control articles were prepared to verify the proper functioning of the test system. The pH of the extracts was measured and the extracts sterile filtered. The maintenance medium on the cell cultures is replaced by the extracts of the test item or control article in triplicate and the cultures are subsequently incubated for 48 hours, at 37±1°C, in a humidified atmosphere containing 5±1% carbon dioxide. Biological reactivity was rated on the following scale: Grade 0 (No reactivity); Grade 1 (Slight reactivity), Grade 2 (Mild reactivity), Grade 3 (Moderate reactivity) and Grade 4 (Severe reactivity). The test item is considered non-cytotoxic if none of the cultures exposed to the test item shows greater than mild reactivity (Grade 2).

RESULTS: No reactivity (Grade 0) was exhibited by the cell cultures exposed to the test item at the 48 hours observation. Severe reactivity (Grade 4) was observed for the positive control article. The negative control article showed no signs of reactivity (Grade 0).

CONCLUSION: Based on the evaluation criteria mentioned above, the test item is considered non-cytotoxic.

RECORD STORAGE: All raw data generated in this study will be archived at Toxikon Europe, according to SOP 4.2.8.

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10. Machineability properties

10.1. Siliconisation

Surface siliconisation is applied to elastomeric closures that are not coated with a polymeric film. The purpose of this siliconisation is to prevent that stoppers start sticking during storage and to guarantee their machineability upon use at the pharmaceutical customer.
The influence of gamma irradiation and subsequent storage on siliconisation is evaluated.
Siliconisation is determined in duplicate by AAS analysis (after extraction with MIBK ; AAS = Atomic Absorption Spectrophotometry).

Table 19 : Siliconisation levels over time after gamma irradiation

Product	Storage Time	0 kGy (µg/cm^2)	25 kGy (µg/cm^2)	40 kGy (µg/cm^2)
V9258 FM480 ISAF2	T = 0m	40.1	39.4	39.1
	T = 3m	48.1	50.0	46.0
	T = 6m	51.0	51.1	48.7
	T = 1 yr	49.0	53.5	54.0

No noticeable difference was observed in siliconisation level at 3 , 6 and 12 months on V9258, FM480 for the various irradiation levels up to 40 kGy.

10.2. Stickiness

This product property was only evaluated on 25 kGy-irradiated products. Products are processed over a conveyor with a feeder bowl.
The products that are sticking together are counted and the result is expressed as % of the total consumed products.

Table 20 : Stickiness over time after gamma irradiation

Product	Storage Time	% Sticking products
V9258 FM480 ISAF2	**T = 0m**	**0.16**
	T = 3m	**0.10**
	T = 6m	**0.19**
	T = 1 yr	**0.05**

For V9258 FM480, a small amount of stoppers are sticking after irradiation up to 25 kGy and a storage time of 1 year. No explicit time evolution is noticed in the results.

11. Ethylene oxide (EtO) sterilizability

Prefillable syringes always use a plunger stopper to assure tightness on the inside of the glass or plastic barrel. Because of their low permeability and because of their chemical cleanliness, plunger stoppers are made from unblended or blended halobutyl (bromobutyl or chlorobutyl) compounds.

On the external side of the syringe either a needle cover or a tip cap are used to assure tightness on the tip of the barrel. It is typical that syringe barrels, pre-assembled with needle covers or tip caps, are sterilized by subjecting them to an ethylene oxide sterilization (EtO) cycle. During such cycle ethylene oxide permeates through the rubber needle cover or tip cap

and then exerts its sterilizing effect. After sterilization, EtO residues are formed that partly are noxious and therefore need to be removed by aeration of the sterilized parts. Particularly aeration permits the removal of EtO itself, but also of ethylene chlorohydrine (ECH).

Needle covers and tip caps are based on elastomers that have a high permeability, such as polyisoprene or styrene butadiene rubber (SBR). Datwyler's FM27 is a styrene-butadiene rubber compound that is well appreciated by the prefillable syringe market, amongst others for its adequate behaviour in EtO sterilization.

Unblended halobutyl compounds have a permeability that is not easily compatible with such an EtO sterilization cycle. FM480 uses a blend of bromobutyl and styrene butadiene rubber polymers and therefore offers a higher gas permeability than unblended halobutyl. This gives an outlook on using one and the same rubber compound, both for the plunger stopper and for the tip cap.

11.1. EtO sterilisation performance of FM480 tip caps

The purpose of the test is to confirm the capability for sterilizing the tip of a syringe by EtO sterilization when the syringe is capped with a tip cap in compound FM480.

Tip caps were assembled on glass barrels in such a way that in the interface between glass and rubber, spores of Bacillus Atropheus were present. A bio-indicator containing approx. 10^6 CFU of Bacillus Atroheus spores is aseptically transferred into sterilized rubber tip caps in FM480, resp. FM27.

Next, these barrels were subjected to a standard ethylene oxide sterilization cycle (705mg/l EtO, 6hrs at 50°C/60%RH).

Growth results on these products were checked. The results of this experiment are given in the table below. Test results certificates are shown in Figure 19 and Figure 20 on the next pages.

Table 21 : Growth count results after EtO sterilisation

Syringe samples	FM480 tip caps Growth results (14 days @ 32 °C)	FM27 tip caps Growth results (14 days @ 32 °C)
10	0/10	0/10
2 positive controls	2/2	2/2

It is confirmed that tip caps in FM480 allow a log 6 reduction of Bacillus Atropheus when treated with EtO, the same way as parts in FM27 do.

Figure 19 : Sterility testing – FM27 after EtO sterilization

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TEST RESULT CERTIFICATE

Amended Report

Project Number:	**TE 08039**	**Study Number:**	**08-B0088-N1AR**
Sponsor:	**Helvoet Pharma**	**Report Date:**	**02/04/2008**
Contact:	*Mrs. Nadia Nouri*	**Amended Report Date:**	**23/04/2008**
Adress:	Industrieterrein Kolmen 1519 3570 Alken Belgium	Technical Initiation: Technical Completion:	18/03/2008 01/04/2008
P.O.Number:	PB0800178		

Study	**Sterility Testing (14 day), Direct Transfer to Test Media**
Test Item	FM27 tip caps: V933[illegible] FM27/0 - [illegible]2-6-13 SAF2
Lot	30060273

REFERENCE: EP 6.0, 2008: 5.1.2: Biological Indicators of Sterilisation
Toxikon SOP 3.1.2.5 Rev: 04: Sterility Test Procedures for Direct Transfer to Test Media
Toxikon SSP 3.20: Product Qualification: Sterility performance by ETO sterilization of syringes capped with FM 480 Tip Caps.

PROCEDURE: Thirteen biological indicator spore strips containing at least a log 6 CFU of *Bacillus atropheus* (ATCC 9372) were each transferred to individual test items. The test items (tip caps) were subsequently placed on accessory syringes and returned to the sponsor. Ten inoculated tip caps on syringes were ETO sterilized at a full cycle by the sponsor. Three tip caps were ETO sterilized at a reduced temperature cycle and served as positive controls. Following the sterilization the biological indicators were returned to Toxikon and transferred aseptically to 10 ml of Trypticase Soy Broth (TSB) and incubated at 33±2°C for 14 days. The test items were examined for signs of growth at days 1, 2, 7 and 14 post inoculation.

RESULTS: The positive controls exhibited growth after 2 days of incubation. No test items sterilized at the full cycle exhibited growth after 14 days of incubation.

CONCLUSION: Sterilizing the tip caps when placed on accessory syringes at a full cycle is sufficient to obtain a log 6 reduction of *Bacillus atropheus* spores within the tip cap.

RECORD STORAGE: All raw data generated in this study will be archived at Toxikon Europe, according to SOP 4.2.8.

AMENDMENT: The reference to SSP 3.19 was changed to 3.20. This amendment has no influence on the integrity of the study.

AUTHORIZED PERSONNEL

ir. Peter Cornelis
Study Director

Vanessa Ruymen
Quality Assurance

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Fortis 230-0391575-06 - KBC 431-0597001-33 - BTW/TVA BE 0442.395.719 - H.R. Leuven 80.154

Figure 20 : Sterility testing – FM480 after EtO sterilization

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TEST RESULT CERTIFICATE

Amended Report

Project Number:	**TE 08039**	**Study Number:**	**08-B0087-N1AR**
Sponsor:	**Helvoet Pharma**	**Report Date:**	**02/04/2008**
Contact:	*Mrs. Nadia Nouri*	**Amended Report Date:**	**23/04/2008**
Adress:	Industrieterrein Kolmen 1519 3570 Alken Belgium	Technical Initiation:	18/03/2008
P.O.Number:	PB0800178	Technical Completion:	01/04/2008

Study	**Sterility Testing (14 days) Direct Transfer to Test Media**
Test Item	FM480 tip caps: V9336 FM480/0 SAF 1
Lot	803906

REFERENCE: EP 6.0, 2008: 5.1.2: Biological Indicators of Sterilisation
Toxikon SOP 3.1.2.5 Rev: 04: Sterility Test Procedures for Direct Transfer to Test Media
Toxikon SSP 3.20: Product Qualification: Sterility performance by ETO sterilization of syringes capped with FM 480 Tip Caps.

PROCEDURE: Thirteen biological indicator spore strips containing at least a log 6 CFU of *Bacillus atropheus* (ATCC 9372) were each transferred to individual test items. The test items (tip caps) were subsequently placed on accessory syringes and returned to the sponsor. Ten inoculated tip caps on syringes were ETO sterilized at a full cycle by the sponsor. Three tip caps were ETO sterilized at a reduced temperature cycle and served as positive controls. Following the sterilization the biological indicators were returned to Toxikon and transferred aseptically to 10 ml of Trypticase Soy Broth (TSB) and incubated at 33±2°C for 14 days. The test items were examined for signs of growth at days 1, 2, 7 and 14 post inoculation.

RESULTS: The positive controls exhibited growth after 2 days of incubation. No test items sterilized at the full cycle exhibited growth after 14 days of incubation.

CONCLUSION: Sterilizing the tip caps when placed on accessory syringes at a full cycle is sufficient to obtain a log 6 reduction of *Bacillus atropheus* spores within the tip cap.

RECORD STORAGE: All raw data generated in this study will be archived at Toxikon Europe, according to SOP 4.2.8.

AMENDMENT: The reference to SSP 3.19 was changed to 3.20. This amendment has no influence on the integrity of the study.

AUTHORIZED PERSONNEL

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11.2. Residual EtO and ECH after EtO sterilization of FM480 products

The requirements for residual EtO (Ethylene Oxide) and ECH (Ethylene ChloroHydrine) are described in ISO 10993-7.
Again, besides FM480, also tip caps in FM27 have been taken up in the evaluation of EtO and ECH desorption after EtO sterilization, considering FM27 as the market reference.
In addition, EtO residuals were followed up in function of aeration time.
Results are given in the 2 figures below:

Figure 21 : Residual Ethylene Oxide upon aeration time

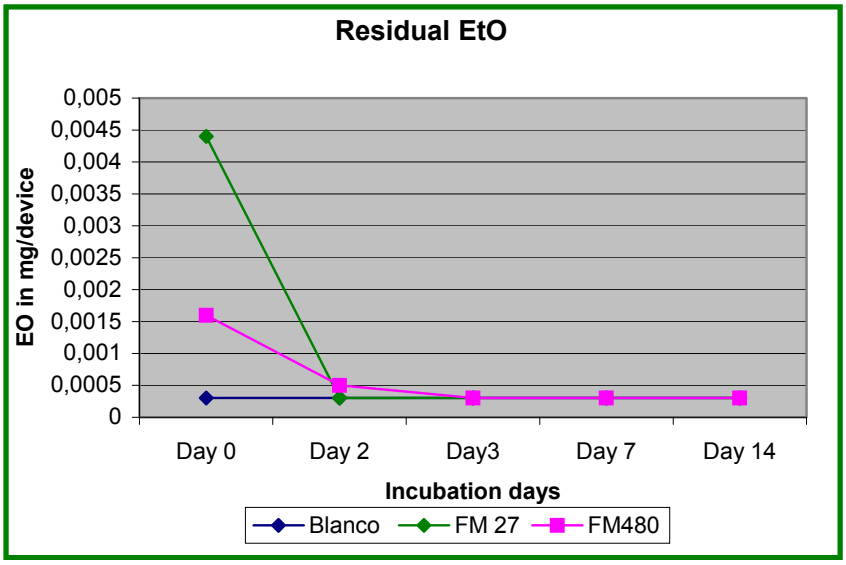
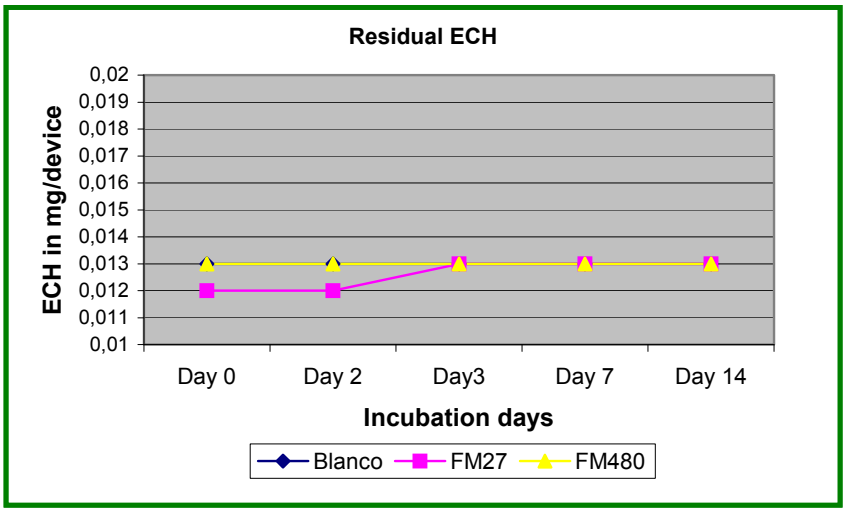


Figure 22 : Residual Ethylene ChloroHydrine upon aeration time



Desorption curves show that FM 480, despite a more limited permeability, is equivalent to FM27 after 3 days of aeration and falls back to values below detection limit.

This time frame is within the typical quarantine time that prefillable syringes are kept after being EtO sterilized.

11.3. Biological properties of FM480 after EtO sterilization

Rubber compound FM480 is non-cytotoxic when tested according to the In-Vitro Cytotoxicity test method of the USP<87> or ISO 10993-5 (see chapter 9 on page 25 in this report).

The same compliance can still be given after an EtO treatment on FM480, followed by usual aeration.

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TEST RESULT CERTIFICATE

Project Number:	**TE 08211**	**Study Number:**	**08-B0511-N1**
Sponsor:	**Helvoet Pharma Belgium NV**	**Report Date:**	**20/03/2008**
Contact:	*Mrs. Nadia Nouri*		
Address:	Industrieterrein Kolmen 1519 B-3570 Alken Belgium	Technical Initiation:	17/03/2008
PO.Number:	PB0800803	Technical Completion:	20/03/2008

Study	**Elution Test - ISO**	Temp/Time	37°C/24 hours
Test Item	V9338 FM480/0 SAP1 Eto	Ratio	25 cm²/20 mL
Lot	Ch803906	Vehicle	MEM-Complete

REFERENCE: Based on "ISO 10993-5, 1999: Biological Evaluation of Medical Devices- Part 5:Tests for In Vitro Cytotoxicity." and "USP 30-NF 25, 2007: <87> Biological reactivity test, in vitro." Toxikon Reference: SOP 3.1.2.3, rev. 05

PROCEDURE: The biological reactivity of a mammalian monolayer, L929 mouse fibroblast cell culture, in response to the test item extract was determined. Extracts were prepared at 37±1°C for 24 hours in a humidified atmosphere containing 5±1% carbon dioxide (static). Positive (natural rubber) and negative (silicone) control articles were prepared to verify the proper functioning of the test system. The maintenance medium on the cell cultures is replaced by the extracts of the test item or control article in triplicate and the cultures are subsequently incubated for 48 hours, at 37±1°C, in a humidified atmosphere containing 5±1% carbon dioxide. Biological reactivity was rated on the following scale: Grade 0 (No reactivity); Grade 1 (Slight reactivity), Grade 2 (Mild reactivity), Grade 3 (Moderate reactivity) and Grade 4 (Severe reactivity). The test item is considered non-cytotoxic if none of the cultures exposed to the test item shows greater than mild reactivity (Grade 2).

RESULTS: No reactivity (Grade 0) was exhibited by the cell cultures exposed to the test item at the 48 hours observation. Severe reactivity (Grade 4) was observed for the positive control article. The negative control article showed no signs of reactivity (Grade 0).

CONCLUSION: Based on the evaluation criteria mentioned above, the test item is considered non-cytotoxic.

RECORD STORAGE: All raw data generated in this study will be archived at Toxikon Europe, according to SOP 4.2.8.

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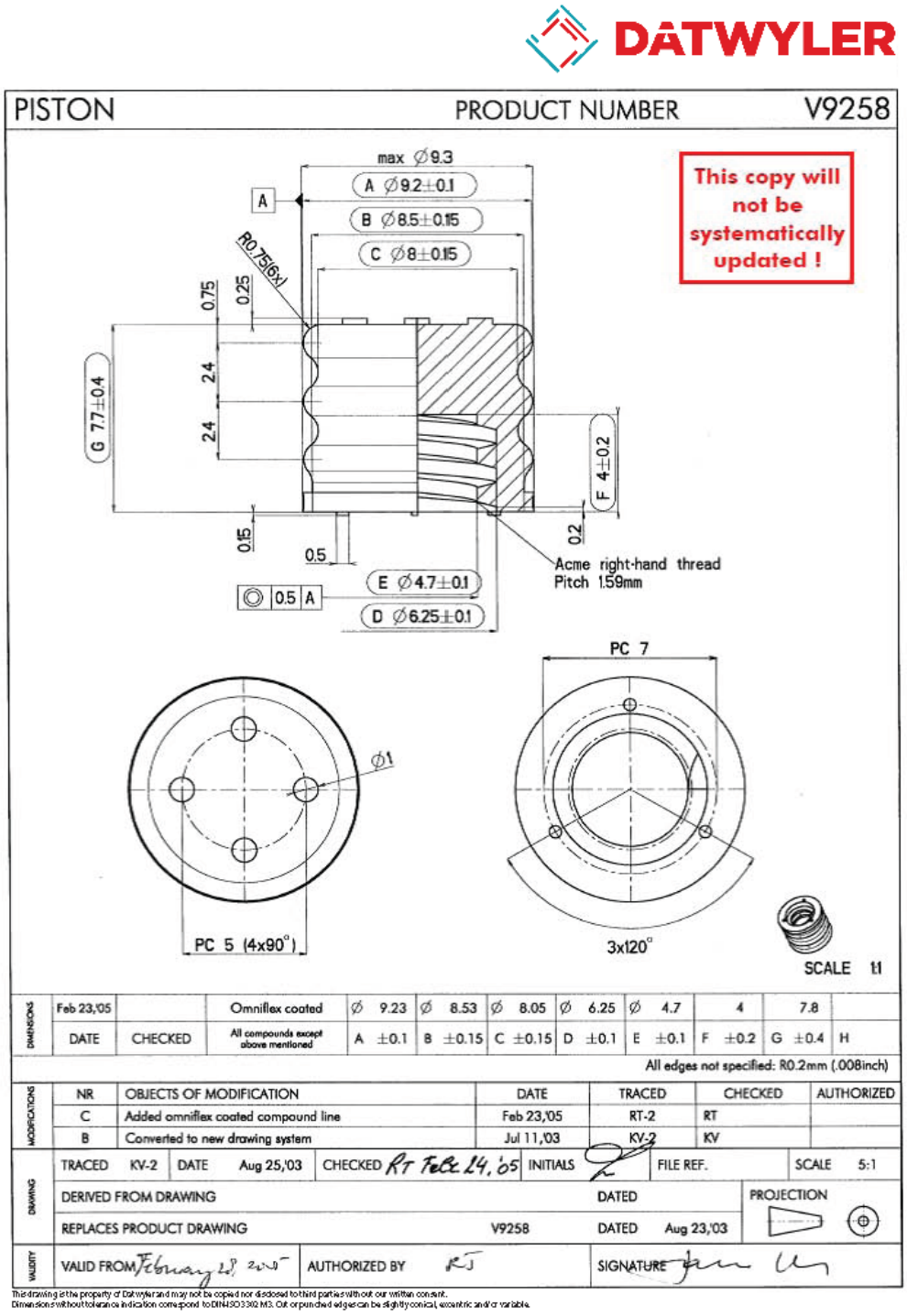
12. Regulatory status

FM480 is recorded in Datwyler Pharma Packaging's USA Inc FDA Drug Master File and in Datwyler's file with the Canadian Health Protection Branch.

On customer request Letters of Authorization can be addressed to FDA or Health Canadian. Your Datwyler Sales representative will be glad to give you support in this.

13. Attachments

13.1. Attachment 1 : 1-3ml Plunger design V9258



13.2. Attachment 2 : tip cap design V9257

DATWYLER

TIP CAP — PRODUCT NUMBER V9257

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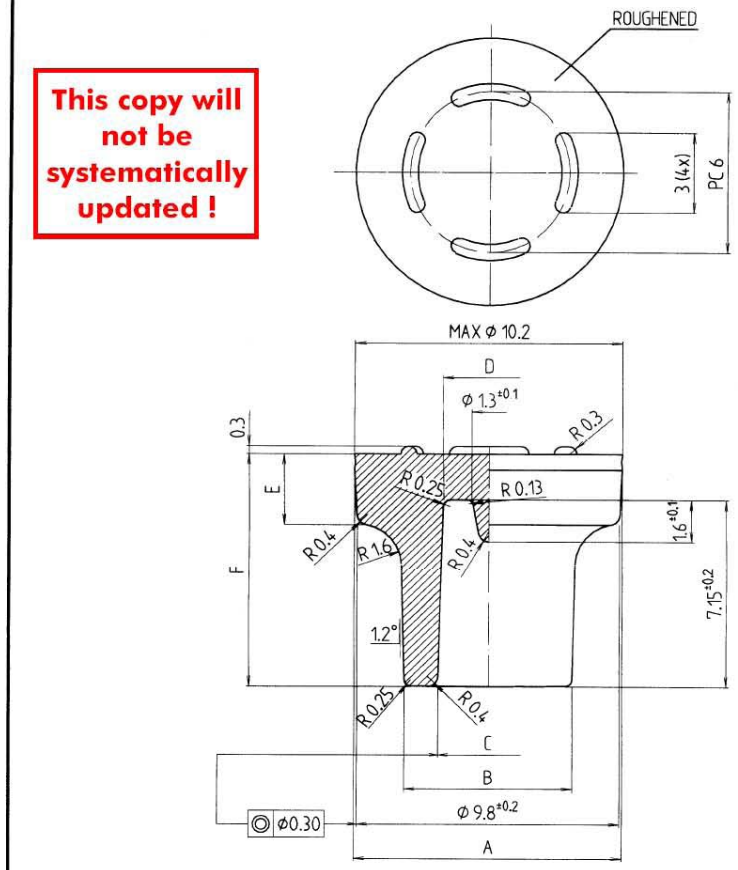

DIMENSIONS & TOLERANCES												
Apr 04.'96		All compounds except above mentioned	Ø 10.0	Ø 6.3	Ø 3,8	Ø 3.4	2.7	8.9		
DATE	CHECKED	FORMULATION	A ±0.2	B ±0.1	C ±0.1	D ±0.1	E ±0.3	F ±0.3	G	H

MODIFICATIONS	OBJECTS OF MODIFICATION	DATE	TRACED	CHECKED	AUTHORIZED

DRAWING					
TRACED FTV-2	CHECKED FTV	INITIALS	FILE REF.	SCALE 5/1	
DERIVED FROM DRAWING DIV1721			DATED Oct 27.'95	PROJECTION	
REPLACES PRODUCT DRAWING			DATED		
VALIDITY	VALID FROM August 5, 1996	AUTHORIZED BY RS	SIGNATURE		

Dimensions without tolerance indication correspond to ISO 3302 and DIN 7715/2. Cut or punched edges can be slightly conical, excentric and/or variable.

14. History

Edition (Issue Date)	*Change (chapter + change)*	*Comment (Rationale)*
2 (October 8, 2009)	Chapter 2: Reference to Health Protection Branch Canada added (#1994-027)	New numbering system at Health Protection Branch Canada
	Chapter 6.1.2.: Rephrasing in paragraph 3	/