National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

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a market surveillance study

RIVM Letter report 2017-0023
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Colophon

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P. Keizers (auteur), RIVM
A. van Drongelen (auteur), RIVM
R. Geertsma (auteur), RIVM
H. Hodemaekers (auteur), RIVM
W. de Jong (auteur), RIVM
E. Lamme (auteur), RIVM
A. Oostlander (auteur), RIVM
B. Roszek (auteur), RIVM
P. Schwillens (auteur), RIVM
B. Venhuis (auteur), RIVM
R. Janssen (auteur), RIVM

Contact:
Peter Keizers
Centre for Health Protection
peter.keizers@rivm.nl

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The Netherlands
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Synopsis

Dermal fillers in the Netherlands
A market surveillance study

Dermal fillers, or just fillers, are products that are injected into or under the skin for medical or cosmetic purposes. This could be to restore the natural contours of the body after an operation for example, but also to mask the visible effects of ageing.

The National Institute for Public Health and the Environment (RIVM) has compiled an overview of 26 so-called non-permanent fillers that were marketed in the Netherlands in 2014, and has analysed these products in a laboratory. The technical files of the 14 manufacturers of these products were also investigated. Following a request through professional associations, 67 treating professionals completed a questionnaire about the fillers that they use and about their potential side effects.

All 26 products from 14 manufacturers proved to be harmless. In order to establish this, an internationally recognised laboratory test that measures harmful effects on cells was carried out. The composition of the products conforms with the description in the technical files. According to the treating professionals, the products from the 14 manufacturers cause very few side effects.

The quality of key sections in the technical files of the 14 manufacturers varied. It is important that manufacturers ensure their technical files are kept in good order. By keeping complete and correct files, manufacturers underpin the safety of the product for the patient, although a limitation in the files does not lead directly to a substandard product. Two sets of files were incomplete, meaning that the safety of the product for the patient is not well substantiated. Most of the inadequacies in the files were of an administrative nature, and are not expected to have any influence on the safety of the product for the patient.

Keywords: fillers, dermal fillers, biocompatibility, product composition, product safety.

Publiekssamenvatting

Rimpelvullers in Nederland

Een onderzoek vanwege markttoezicht

Rimpelvullers, of fillers, zijn producten die in of onder de huid gespoten worden met een medisch of cosmetisch doel. Dit kan bijvoorbeeld zijn om de natuurlijke lichaamsvorm te herstellen na een operatie, maar ook om de zichtbare gevolgen van ouder worden te maskeren.

Het RIVM heeft een overzicht gemaakt van 26 zogeheten niet-permanente fillers die in 2014 in Nederland op de markt waren en deze in een laboratorium geanalyseerd. Ook zijn de technische dossiers van de 14 fabrikanten van deze producten onderzocht. Na een verzoek hiertoe via beroepsverenigingen hebben 67 behandelaars een enquête ingevuld over de fillers die zij toepassen en over mogelijke bijwerkingen.

Alle 26 producten van 14 fabrikanten blijken niet schadelijk te zijn. Hiervoor is een internationaal erkende laboratoriumtest uitgevoerd die schadelijke effecten op cellen meet. De samenstelling van de producten komt overeen met de beschrijving in het technische dossier. De producten van de 14 fabrikanten veroorzaken volgens behandelaars weinig bijwerkingen.

De kwaliteit van belangrijke onderdelen van de technische dossiers van de 14 fabrikanten varieerde. Met volledige en correcte dossiers onderbouwen fabrikanten de veiligheid van het product voor de patiënt, maar een beperking in het dossier betekent niet direct een minderwaardig product. In een tweetal gevallen vertonen de dossiers onvolledigheden waardoor de veiligheid van het product voor de patiënt niet goed onderbouwd is. De meeste tekortkomingen in de dossiers zijn van administratieve aard en hebben daarmee naar verwachting geen invloed op de veiligheid van het product voor de patiënt. Het is van belang dat de fabrikanten er voor zorgen dat hun technische dossiers op orde zijn.

Kernwoorden: fillers, rimpelvullers, biocompatibiliteit, productsamenstelling, productveiligheid.

Contents

Summary — 9

1 Introduction — 11
1.1 Background — 11
1.2 Aim of the study — 12
1.3 Guide to reading the report — 13

2 Market survey — 15
2.1 Scientific literature — 15
2.2 Dermal fillers applied in the Netherlands in 2014 — 15
2.3 Products selected for the market surveillance study — 18

3 Assessment of the technical documentation — 19
3.1 Overall assessment of the documentation — 20
3.2 Instructions for use — 20
3.3 Risk analysis — 21
3.4 Biocompatibility — 21
3.5 Physical testing — 21
3.6 Clinical evaluation — 21
3.6.1 Equivalence — 22
3.6.2 Claims — 22
3.6.3 Safety and performance analysis — 22
3.7 Summary and analysis of PMS data — 22
3.8 Potential impact of findings on patient safety — 22
3.8.1 IFU — 23
3.8.2 Risk analysis — 23
3.8.3 Biocompatibility — 23
3.8.4 Clinical evaluation — 23
3.8.5 PMS data — 23
3.9 Discussion and conclusions — 24

4 Physicochemical analysis — 25
4.1 Identity of hyaluronic acid-based fillers — 25
4.2 Cross-linking grade of hyaluronic acid-based fillers — 26
4.2.1 Comparison to technical files — 28
4.3 Identity of non-hyaluronic acid-based fillers — 29
4.3.1 Comparison to technical files — 32
4.4 Discussion and conclusions — 32

5 Biocompatibility — 35
5.1 Cytotoxic activity — 35
5.2 Discussion and conclusions — 39

6 Overall discussion and conclusions — 41

References — 41

Annex 1: Request of IGZ — 45

Annex 2: Methods of assessment of technical documentation — 49

Annex 3: Request of IGZ — 51

Annex 4: Checklist for request Dutch fillers — 53

Annex 5: Assessment form — 55

Annex 6: Results of the technical documentation assessment — 64

Annex 7: Products received in market surveillance — 68

Annex 8: Physicochemical methods — 70

Annex 9: Biocompatibility methods — 72

Annex 10: Results of the biocompatibility screening — 74

Summary

In this study, we have assessed the technical files and analysed product samples from 14 manufacturers marketing dermal fillers in the Netherlands.
The following five questions were addressed:

1. Which non-permanent dermal fillers are being used in the Netherlands?
2. Do the technical files of the selected non-permanent dermal fillers provide adequate proof of conformity with the requirements of the Medical Devices Directive?
3. Are key physicochemical characteristics of the products, such as material identity and degree of cross-linking, in line with the information in the technical documentation?
4. As part of a biocompatibility evaluation, does the material as present in the products show potential toxicity by leaching of toxic compounds?
5. In case of shortcomings, do these lead to a concern for patient safety?

As a general conclusion, several key physicochemical and biocompatibility characteristics of the products as determined in the laboratory analysis were found to be good. On the other hand, the technical documentation contained shortcomings at some aspects. Complete and correct documentation is the basis to warrant patient safety. Although the potential impact on patient safety of the particular shortcomings found in the files is expected to be limited, they should be carefully considered and resolved by the manufacturers in order to substantiate the quality and safety of their products as required in the regulatory system.

1 Introduction

1.1 Background

Soft tissue fillers, also known as injectable implants, dermal fillers or wrinkle fillers, are implants primarily used for filling of rhytides (skin wrinkles) and folds, as well as correction of volume loss and augmentation of the aging face [1]. In the early 1980s, bovine collagen was introduced as the first injectable filler approved by the Food and Drug Administration (FDA) for cosmetic injection [2]. With the increasing desire for a youthful appearance among the aging population, industry has responded by increasing the number of available treatment options to meet the demands of the population As such, filler materials used today are composed of a wide range of substances including collagen, hyaluronic acid, calcium hydroxylapatite, poly-L-lactic acid, and other synthetic or manmade polymers. In the US, about 25 filler products are approved for dermatologic indications, each with its own properties, advantages, and disadvantages [1]. In Europe, there are over 140 dermal fillers on the market [3].

Fillers can be categorised as either permanent or non-permanent (including temporary and semi-permanent fillers). Permanent fillers are made of non-biodegradable material that will stay in the human body after injection as it is not absorbed. Such products may contain polymethyl methacrylate microspheres, highly purified forms of liquid silicone, and hydrogel polymers [4]. Non-permanent fillers are naturally occurring substances and include for example hyaluronic acid, collagen, or hydroxylapatite. These materials will stay in the body for a certain time, but are eventually absorbed.

Clinical experience has shown that fillers must be used with caution as complications can occur [5]. Complications can be treatment-related or product-related and reactions can occur immediately or delayed and show both short-term and long-term duration. The time until an adverse reaction occurs as well as the type of adverse reaction vary between different fillers [5, 6]. In a report from the Injectable Filler Safety Study, a German-based registry for adverse filler reactions, adverse reactions to non-permanent fillers were reported to occur after 4.9 ± 5.8 months and reactions to permanent fillers after 18.3 ± 19.0 months. Adverse reactions to hyaluronic acid-based fillers were mainly swelling, erythema and nodules, while poly-L-lactic acid and polymethylmethacrylate fillers caused the development of granulomas [6]. In a European survey, permanent fillers were responsible for severe, persistent, and recurrent adverse effects [7].

Not only the type of filler (permanent vs. non-permanent) but also the inherent properties of the product correlate with the occurrence of adverse reactions. For example, the longevity of hyaluronic acid-based products depends amongst others on the concentration of the product and the level of cross-linking [8]. Naturally occurring hyaluronic acid is rapidly degraded with a half-life of only 12 to 24 hours. Cross-linking hyaluronic acid increases its tissue residency and elasticity. The degree

of cross-linking enhances the persistence of the filler by increasing the resistance to degradation by native hyaluronidase [8]. However, this may also reduce its biocompatibility, causing foreign body reaction and encapsulation [9].

In 2012, the Dutch Health and Youth Care Inspectorate (IGJ, previously the Dutch Health Care Inspectorate) was informed by the Dutch Association of Cosmetic Healthcare (NVCG) of adverse events after injections with Hyacorp, a cross-linked hyaluronic acid filler with large particles. Most of the complaints concerned hardness and (excessive/recurrent) swelling [10]. These complaints also appeared after treatment with hyaluronidase whether or not combined with an anti-inflammatory agent. Therefore, the biodegradability of Hyacorp fillers was questioned. In the subsequent investigation performed by RIVM [10], it was concluded that it may take a very long time before strongly cross-linked fillers, such as Hyacorp fillers, are completely degraded and absorbed by the body. A possible explanation for the observed adverse events was that the modification grade/degree of cross-linking was so high that the enzyme did not recognize the hyaluronic acid which led to foreign body reactions. Based on the investigation, the Inspectorate subsequently removed the product Hyacorp from the market [11].

The Inspectorate is entrusted with market surveillance and law enforcement of medical devices and their use in order to warrant patient safety. Until today, safety and tolerability of dermal fillers are not fully understood. It is largely unknown if differences between fillers explain why one product leads to adverse events and another does not. Therefore, the Inspectorate asked for a market surveillance study on dermal fillers on the Dutch market in 2014. The use of permanent dermal fillers for aesthetic purposes is prohibited in the Netherlands (2015/C 241/01), as the risk of complications does not outweigh the benefits in this situation. Consequently, this study focusses on non-permanent dermal fillers.

1.2 Aim of the study

The aim of this study is to investigate non-permanent dermal fillers available on the Dutch market. In order to do this, we have addressed the following questions:

1. Which non-permanent dermal fillers are being used in the Netherlands?
2. Do the technical files of the selected non-permanent dermal fillers provide adequate proof of conformity with the requirements of the Medical Devices Directive (MDD) [12]?
3. Are key physicochemical characteristics of the products, such as material identity and degree of cross-linking, in line with the information in the technical documentation?
4. As part of a biocompatibility evaluation, does the material as present in the products show potential toxicity by leaching of toxic compounds?
5. In case of shortcomings, do these lead to a concern for patient safety?

1.3 Guide to reading the report

In the following chapter the results of the market survey are presented as well as the products selected for further study. In Chapter 3 the results of the assessment of the technical files are described. Chapter 4 shows the results of the physicochemical analyses. The biocompatibility results are presented in Chapter 5. Finally, the overall results are discussed and general conclusions are presented.

2 Market survey

2.1 Scientific literature

To investigate which non-permanent dermal fillers are currently available, a literature search was performed. Literature was searched in PubMed using the search term 'soft tissue fillers', with the restrictions that the publication should be a review publication, published between January 1st 2013 and August 28th 2015, and based on studies in humans. In addition, an internet search was performed in Google using the search terms 'soft tissue fillers', 'semi-permanente rimpelvullers', and brand names collected from the publications obtained from the PubMed search. The products identified are summarized in Table 2.1. This literature search is an update of a search on non-permanent dermal fillers performed in 2007 [13]. The products identified at that time can also be found in Table 2.1.

2.2 Dermal fillers applied in the Netherlands in 2014

To investigate which non-permanent dermal fillers are applied in the Netherlands, a questionnaire was made intended for users of the products. A copy of the letter accompanying the questionnaire as sent by the Dutch Health and Youth Care Inspectorate (IGJ) can be found in Annex 1. The questionnaire included the following questions:

- which non-permanent fillers do you use for aesthetic purposes (brand, series/type, main constituent, and supplier),
- how often did you use the product(s) in 2014, and
- which product(s) led to adverse reactions in the past 3 years.

The questionnaire was sent to all associations of health care professionals who are likely to perform treatments with soft tissue fillers. All these associations belong to the 'Nederlandse Stichting Esthetische Geneeskunde', and include:

- 'Nederlandse Vereniging voor Keel- Neus- Oorheelkunde' (NVKNO)
- 'Nederlandse Vereniging voor Cosmetische Geneeskunde' (NVCG)
- 'Nederlandse Vereniging voor Cosmetische Chirurgie' (NVVCC)
- 'Nederlandse Vereniging voor Dermatologie en Venereologie (NVDV)
- 'Nederlandse Vereniging voor Mondziekten, Kaak- en Aangezichtschirurgie' (NVMKA)
- 'Nederlands Oogheelkundig Gezelschap' (NOG).
-

In addition, the questionnaire was sent to the 'Nederlandse Vereniging voor Plastische Chirurgie' (NVPC) as well as the 'Nederlandse Vereniging voor Esthetische Plastische Chirurgie' (NVEPC). The membership base of these 8 associations is unknown.

In total, 67 responses were received: 36 respondents were member of the NVPC, 14 of the NVCG, 1 of the NVKNO, 1 of the NVDV, and 1 of the NVMKA. Fourteen respondents did not mention to which association they

belonged. In total, 18 of the 67 respondents reported not to use dermal fillers.

Table 2.1. Overview of non-permanent dermal fillers identified by literature search

Material	Product name	Manufacturer, Country
Cross-linked hyaluronic acid	Juvederm Ultra, Volbella[1] [14]	Allergan, USA
	Belotero[2] [14]	Merz Pharma GmbH, Germany
	Glytone[*3] [14]	Pierre Fabre, France and Merz Pharma GmbH, Germany
	Teosyal[4] [14]	Teoxane SA, Switserland
	Prevelle Silk[5] [15]	Mentor Worldwide LLC, USA
	Emervel[6] [15]	Galderma, Switserland
Cross-linked alginate	Novabel Derma Filler[**] [16]	Merz Pharma GmbH, Germany
Hyaluronic acid with human mesenchymal cells	Injectable tissue-engineered soft tissue [17]	Korea University Guro Hospital, Korea
Cultured autologous skin cells with collagen	LAVIV (azficel-T)[7]	Fibrocell Science, USA
Cross-linked hyaluronic acid	ELEVESS[***]	Anika Therapeutics, USA
	Esthelis Soft, Basic, Men	Anteis SA, Switserland
	ISOGEL Class 1, 2, 3	Filorga, France
	Juvederm 18, 24, 24HV, 30, 30HV	Leaderm/Corneal, France
	Restylane, Perlane, Touch, Lipp, SubQ	Q-med, Sweden
	Surgiderm 18, 24, XP, 30XP, Surgilips, Surgilift PLUS	Leaderm/Corneal, France
	Visagel	SurgicalConcepts GmbH, Germany
Sephadextran hyaluronic acid	Reviderm intra	Rofil, the Netherlands
Dissolved polyacrylamide hydrogel	Beautical 2, 5[****]	ProCytech, France
Carboxymethylcellulose and polyethylene oxide	Laresse	FzioMed Inc, USA
Polyvinyl alcohol 8%	Bioinblue	Polymekon, Italy
Calcium hydroxylapatite	Radiesse	BioForm Medical Inc, USA

Grey-shaded products are identified by the literature search in 2015, the other products are taken from the search in 2007 [13].
[*] Product has been renamed Etermis since November 2015.
[**] Product has been withdrawn from the market due to serious adverse reactions: https://www.gov.uk/drug-device-alerts/medical-device-alert-novabel-dermal-filler-practitioners-should-stop-use-and-return-all-unused-products.
[***] ELEVESS was introduced to the European market in 2007, however, the brand is not mentioned by the professional users in the current market survey.

**** Products have been withdrawn from the market due to non-conformities regarding the manufacturing process of the products.
Internet references: [1] http://www.juvederm.com; [2] https://global.belotero.com;
[3] http://www.merzaesthetics.eu//nl/products/glytone/index.jsp; [4] http://www.teosyal.nl;
[5] http://www.mentorwwllc.com/global-ca/Face.aspx; [6] http://www.emervel.nl;
[7] http://www.dermalfillersreview.com/laviv.

The respondents reported the use of 96 dermal fillers from 13 different brands of non-permanent dermal fillers, see Figure 2.1. Some respondents reported on the use of just one specific type of filler belonging to one of these brands, while other respondents reported on the use of all available types of fillers of a brand. Overall, the most often mentioned brands are Juvederm, Restylane and Radiesse.

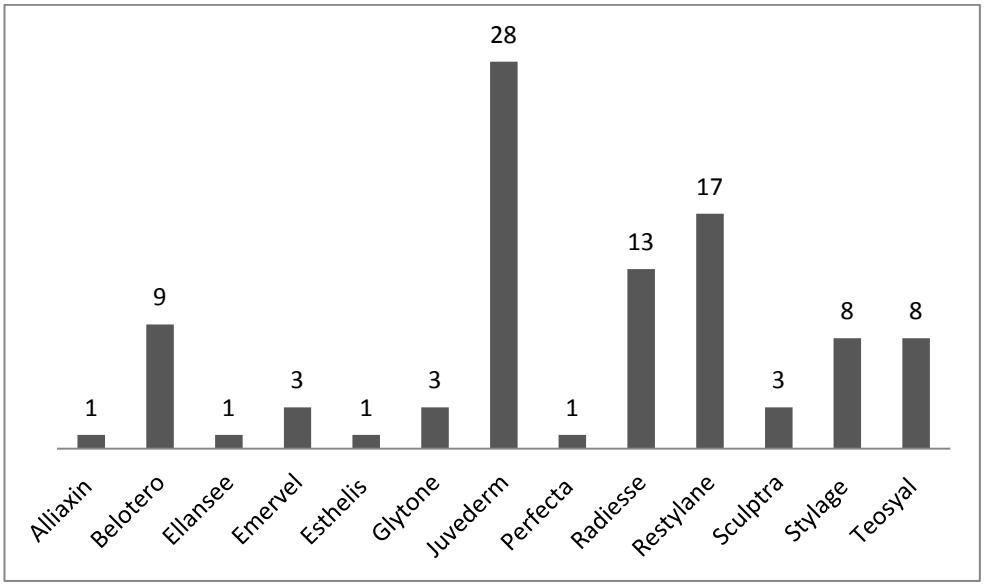

Figure 2.1. The used dermal filler brands in the Netherlands in 2014, as reported by the 67 respondents. The number above each bar represents the number of respondents who reported on the use of the particular brand.

The main constituent of the dermal fillers was generally hyaluronic acid: in 78 out of 96 fillers. Thirteen fillers consisted mainly of calcium hydroxylapatite, four fillers of poly-L-lactic acid and one filler of poly-ε-caprolacton. For the 13 mentioned brands, 14 manufacturers have been reported. Frequently mentioned manufacturers were Allergan (n=26), Galderma (n=20) and Merz (n=20).

In total, 17,169 treatments with non-permanent dermal fillers were reported in our survey. The most often applied brands were Juvederm, Restylane and Emervel, see Figure 2.2.

Fifty-four adverse reactions were reported. The mentioned adverse reactions ranged from no effect and temporary swelling to allergic reactions and infections. No severe adverse reactions were reported. The small number of adverse reactions reported in the questionnaire may either indicate that adverse reactions do rarely occur or that adverse reactions are underreported.

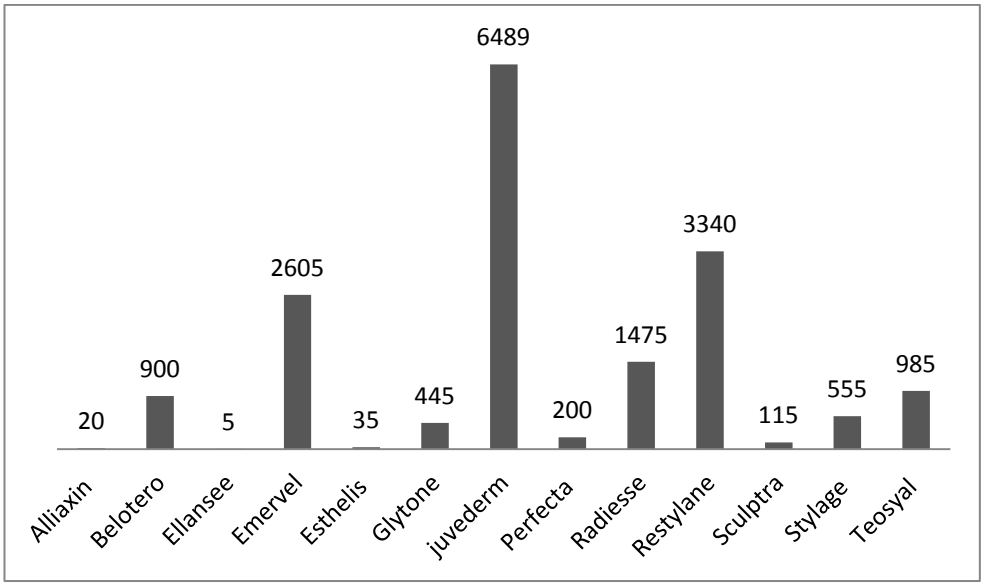

Figure 2.2. The number of treatments performed with a certain dermal filler brand in the Netherlands in 2014 as reported by 67 respondents. Sometimes respondents reported one number for several products. In that case, the number of treatments was divided by the number of products reported by the respondent and subsequently equally distributed over the products. Sometimes respondents did not report on numbers. In that case, data are missing.

It should be noted, that the total number of respondents approached by the associations which the Inspectorate contacted is unknown. However, since only 67 respondents filled out the questionnaire, this appears to be only a small selection of the respondents approached. Therefore, the numbers reported in this section may not be representative of all appliers of fillers in the Netherlands.

2.3 Products selected for the market surveillance study

The questionnaire provides an indication for the list of dermal fillers used in 2014, though it might not be exhaustive. Information from another, concurrent dermal filler project was available which might add to ours. Therefore, we requested our partners from that project for a list of products that were used in 2014 according to their knowledge. From this list, one additional brand was identified, namely Princess. In total, this led to 14 brands of non-permanent dermal fillers being selected for the market surveillance study. For composition and biocompatibility analysis, two product types of each brand were selected, when more than one type was available. In case adverse events were reported in the COEN database, the first selected product type was the product type with the highest number of records. Additionally, a second product with no or the least number of records was selected as well. Of each brand, the technical file of the product type with the highest level of hyaluronic acid or cross-linking was analyzed. In total, 26 products were selected for composition and biocompatibility analysis.

3 Assessment of the technical documentation

In order to show compliance with the MDD [12], manufacturers of medical devices have to compile a technical file. A predefined selection of the technical documentation was requested from the manufacturers for assessment. The documentation received often dealt with several types of dermal fillers. In these cases, the documentation related to one of the fillers was chosen for assessment. The method used for assessment of the documentation is described in detail in Annex 2.

In short, a form was developed in order to enable a structured and uniform assessment of the files (see Annex 5). The form consisted of file items (e.g. risk analysis), which were in turn subdivided into sub-items (e.g. risk management plan). For every sub-item, presence of adequate information was scored with yes/no/partial, or similar scoring options as relevant to the particular sub-item. The scoring system discerned sub-items of normal and major importance in relation to risk and safety aspects (see Annex 5), resulting in a higher weight and consequently higher score for major sub-items. The overall score for file items was obtained as the sum of the sub-item scores. The sum translated into a ‘good’, ‘moderate’ or ‘insufficient’ score. Importantly, failure for one major sub-item immediately led to an insufficient score for the file item as a whole. This type of scoring system has been used before in previous RIVM file assessment projects [18, 19].

All manufacturers of the 14 dermal filler brands provided the requested technical documentation. One of the manufacturers indicated that he was not the original manufacturer, but had an agreement to sell products of the original manufacturer under its own name (this kind of agreement is usually referred to as “own brand labeling”). As part of the agreement, the manufacturer also had access to the technical documentation of the original manufacturer. Since both products were selected for this study and the two files were largely identical, only 13 technical files were assessed.

After assessment, manufacturers were informed about the results and were given the opportunity to respond to the findings. In case a manufacturer believed the assessment score of a specific item contained factual inaccuracies, the manufacturer was allowed to either state were the specific information could be found in the original submitted documentation or provide additional documentation which contained the specific information. In the latter case, only documentation dated from before February 2016, the date of initial information request, was considered.

The following paragraphs summarize the anonymized results of the technical documentation assessment, starting with an overview of the overall findings per dermal filler. The subsequent paragraphs describe the findings per documentation item in more detail. The complete results of the technical documentation assessment are presented in Annex 6. At the end of this chapter, an evaluation is given of the

potential impact on patient safety of shortcomings found in the documentation files.

3.1 Overall assessment of the documentation

The assessment score varied considerably per dermal filler documentation set, see Figure 3.1. Initially none of the documentation sets was entirely 'good', 'moderate', or 'insufficient'. The manufacturers of DF06, DF09, DF11 and DF12 provided additional information for assessment. Thereafter, the documentation set of DF06 scored entirely 'good'. DFO4 and DF10 scored three times as insufficient, DFO5, DFO7, and DF11 scored as insufficient once and DFO1, DFO2, DFO3, DF06, DF08, DF09, DF12 and DF13 did not score any insufficient. The manufacturers of DF02, DF07, DF11 and DF12 commented that they have updated their documentation sets after February 2016. Since only information from before February 2016 was taken into account, these updates were not assessed in the current study. The items summary and analysis of PMS data most often scored 'good', while clinical evaluation most often scored 'insufficient'. However, it should be realized that Clinical evaluation had more sub-items than PMS data (15 and 4 sub-items respectively). Therefore, clinical evaluation is more likely to yield submaximal scores. Furthermore, it should be noted that, while it is important that the technical documentation is providing all the necessary information in the correct section of the file, shortcomings in the file do not necessarily have impact on patient safety. As discussed at the end of this chapter, the potential impact on patient safety of the observed shortcomings in this study is expected to be limited.

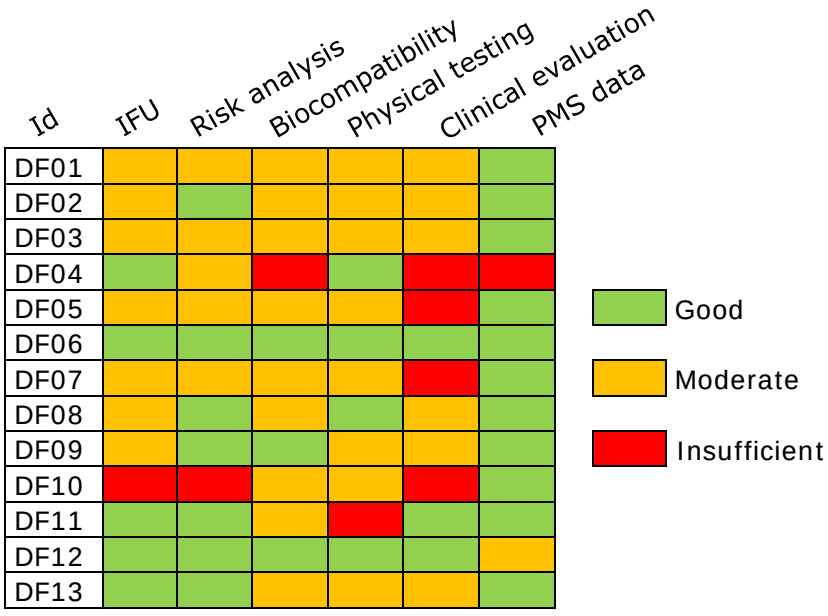

Figure 3.1. Results of the assessment of technical documentation
Abbreviations: DF – dermal filler; Id – identifier; IFU – instructions for use; PMS data – Summary and analysis of Post Market Surveillance data

3.2 Instructions for use

The instructions for use (IFU) assessed were all either in Dutch or in English, which are both languages allowed for professional users of a

medical device in the Netherlands. All IFUs included the sub-item indications for use. The four required categories of dermal filler-related risks were also addressed in all IFUs. However, the major sub-item injection technique was not addressed in one IFU and partially in seven files. Although not submitted with the IFU, several files referred to a specific leaflet on the details for injection of the dermal filler for the healthcare professional. However, such important information should be an integral part of the IFU [12].

3.3 Risk analysis

All risk analyses contained a risk management plan and a recent date/version number. Moreover, the risk control/mitigation as well as the acceptability of the residual risks were also addressed in all files. The dermal filler-related risks were only partially described (e.g. mentioning contra-indications in general without specifying them) in one file. The same file did not give a risk estimation and also a conclusion section was missing. In half of the documentation files, all required general risk categories, based on hazards derived from the standard for risk management of medical devices ISO 14971 [20], were addressed. Examples of categories that were missing in the other files are biocompatibility, chemical hazards and disposal.

3.4 Biocompatibility

A literature review is considered essential as a first step in a biological evaluation [21]. This is required in order to take account of the existing knowledge and the generally acknowledged state of the art, regarding the evaluation of biocompatibility of particular products. Furthermore, the review is used to prevent unnecessary animal tests being performed. In only two files such a literature review was performed adequately. The appropriateness of the tests conducted was not adequately addressed in four files: in three files only a reference to the relevant standard was provided without further explanation and in one file the appropriateness of the tests was not addressed at all. All files included information on the tests conducted, the standards applied and the test protocols used. In one file, a summary of the results and a conclusion section were absent, because the submitted documentation consisted of only test reports.

3.5 Physical testing

There is no standard pertaining to physical testing of dermal fillers. In general however, rheology tests (e.g. determining the elastic and viscous modulus of a filler) and the extrusion force test (e.g. evaluation of injectability of a filler) are considered to be good indicators of the physical properties of fillers. All files contained information on the physical tests that were performed, with rheological testing and extrusion force testing performed most frequently. In half of the files, the appropriateness of the physical testing performed was only partially addressed. A summary of results and/or a conclusion section were not covered in two files.

3.6 Clinical evaluation

The clinical evaluation is an extensive item in the technical documentation file and comprises 15 sub-items (see Table 6.5 in Annex

6). The results on major sub-items as well as the most remarkable findings on normal sub-items are addressed below.

3.6.1 Equivalence

In case the characteristics of two medical devices are similar to a large extent (i.e. equivalent), it can be assumed that there would be no clinically significant difference in their safety and performance. Consequently, the so-called equivalence principle, can be used, which means the clinical data of one device can be used in the clinical evaluation of the other device without conducting a new clinical investigation. However, this principle can only be used if literature provides strong evidence. In addition, clinical, technical, and biological characteristics of the two products should be included in the demonstration of equivalence according to the MEDDEV guidance document on clinical evaluation [22]. In six files, a rationale to substantiate the equivalence contained the required elements, whereas in two files the substantiation was only partially addressed. Equivalence was not claimed for the remaining six files.

Most clinical evaluation reports included clinical evidence based on clinical investigations, literature data and sometimes PMS data, in combination with the equivalence principle. If the equivalence principle was applied, the dermal filler was compared with products from the same manufacturer and/or with those of competitors. Similarities and differences of dermal filler characteristics were listed with varying levels of detail and completeness.

3.6.2 Claims

Only one sub-item, namely safety and performance claims, did not meet the requirements in more than half of the files: the item was not included in six files and partially included in three files.

3.6.3 Safety and performance analysis

The major sub-items ‘performance analysis’, ‘safety analysis’ and ‘presence of relevant topics in the clinical evaluation’ were partially addressed in two files and adequately addressed in all other files. The last major sub-item, summary of clinical data and appraisal, was adequately addressed in approximately half of the files, whereas this item was partially addressed in the other half.

3.7 Summary and analysis of PMS data

Identification of PMS sources was the only sub-item of the summary and analysis of PMS data that was well addressed in all files. The actual analysis of PMS data, the summary of PMS data/conclusions and actions to be taken were not addressed in one file. In two other files, the summary of PMS data/conclusions was partially addressed.

3.8 Potential impact of findings on patient safety

This paragraph analyses to what extent the findings described above may affect patient safety. Shortcomings in the technical documentation could imply that product safety and safe use of the device are insufficiently guaranteed. This in turn could have impact on patient safety. On the other hand, the impact of shortcomings could be

counterbalanced by available information in other parts of the file, the file could be poorly maintained while the device is of high quality, or the manufacturer could have omitted to provide crucial parts of the documentation. Thus, while it is important that the technical documentation is providing all the necessary information in the correct section of the file, shortcomings in the file do not necessarily have impact on patient safety.

3.8.1 IFU

Depending on the knowledge and expertise of health care professionals involved, inadequate information on the injection techniques in the IFU could have an impact on patient safety, as has been shown for Hyacorp fillers [10]. Most IFUs acknowledged the importance of application methodology and as such stated that the user should be an adequately trained and qualified health care provider. While they did not actually include this information in the IFU or in the technical file, some technical files referred to an additional leaflet specifically on injection techniques. Therefore, the actual impact on patient safety of this shortcoming in the IFU is uncertain. In the hands of an adequately trained and experienced user there will only be a small potential impact on patient safety.

3.8.2 Risk analysis

For the risk analysis, the most frequently observed shortcoming was the absence of several dermal filler specific and general risks. When not all relevant risks are analyzed, important measures to mitigate these risks may be missed, which in turn may pose a risk for the patient.

3.8.3 Biocompatibility

The moderate and insufficient scores obtained for biocompatibility are primarily caused by the absence of a literature review and insufficient substantiation for the appropriateness of the tests to be performed. However, in all cases, a standard set of tests was performed according to applicable standards and the results did not indicate problems. Consequently, the potential impact on patient safety of the shortcomings for biocompatibility is counterbalanced by the data from testing and the shortcomings are expected to have a negligible impact on patient safety.

3.8.4 Clinical evaluation

Clinical evaluation is critical for the evaluation of safety and performance of the dermal fillers and information in the technical file should be updated to current standards. However, an analysis of the shortcomings leading to the ‘moderate’ and ‘insufficient’ scores for this file item showed that they will have a relatively low potential impact on patient safety. All of the major sub-items were at least partially present. Furthermore, information that was missing in the various sub-items was judged to be counterbalanced by information in other sub-items in the same file.

3.8.5 PMS data

In one file, no analysis or summary of PMS data was present and no actions were taken related to PMS data. As a consequence, if indeed not carried out, the possibility to implement necessary actions or the opportunity to improve the functionality of the product may be missed, which is judged to have potential impact on patient safety. Two other

files had a limited summary, which is not expected to have significant impact on patient safety. In the other files no shortcomings were observed for this item.

3.9 Discussion and conclusions

The content of the technical documentation varied considerably between products. Given the fact that in the regulatory system for medical devices the quality and safety of products is required to be substantiated by the information in the files, this outcome should be reason for manufacturers to make improvements in their files.

Although it is important that the technical documentation is providing all the necessary information in the correct section of the file, shortcomings in the file do not necessarily mean that the device is of insufficient quality. An analysis of the shortcomings showed that of most shortcomings the potential impact on patient safety can be considered limited since these have a more administrative character. However, DF04 scored insufficient on PMS data and DF10 scored insufficient on risk analysis. In these two cases, the shortcomings could imply that product safety and safe use of the device are insufficiently guaranteed.

In conclusion, shortcomings were observed in varying numbers in the technical files. Although their potential impact on patient safety in general may be limited, they should be carefully considered and resolved by the manufacturers in order to substantiate the quality and safety of their products as required in the regulatory system.

4 Physicochemical analysis

All 26 products selected for physicochemical analysis were supplied by the manufacturers. Since products were provided in duplicate or triplicate, a total of 75 samples were received. Detailed information on specifications of the products can be found in Annex 7. Sixty-four samples are based on hyaluronic acid, representing 22 different dermal fillers. Six samples are based on poly-ε-caprolacton and represent two different dermal fillers. Two samples are based on poly-L-lactic acid, representing one type of dermal filler. And lastly, three products are based on hydroxylapatite and also represent one type of dermal filler. All 75 samples but one were analysed. All hyaluronic acid-based fillers were analysed for identity and cross-linking grade. The other fillers were analysed for elemental composition (max. two batches per filler) and particle size.

4.1 Identity of hyaluronic acid-based fillers

The identity of the fillers was determined by both LC-MS and NMR spectroscopy, after an enzymatic digestion of the product. The details of the methods are described in Annex 8. Hyaluronic acid is a polymer of a disaccharide repeating unit, see Figure 4.1. The polymer can be broken down in smaller fragments by lyase-type of enzymes (Figure 4.1).

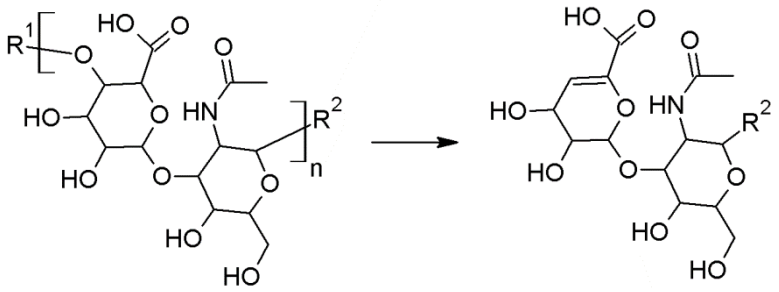

Figure 4.1: Structure of hyaluronic acid (HA, left) with the repeating unit between brackets. The reaction shown represents the β-(1-4) lyase activity of chondroitinase AC, leading to a fragment containing an unsaturated bond in the glucuronic acid (right). R^1 and R^2 represent HA repeating units or the terminal alcohols.

All of the investigated samples that were marketed as hyaluronic acid-based dermal filler were confirmed to be composed of hyaluronic acid. Hyaluronic acid was not found in two products marketed as poly-ε-caprolacton-based dermal filler (samples A097419 to A097424), which was in agreement with their specifications. In accordance with the technical files, all filler types but one (sample A098307) were found to contain a cross-linker. In all cases, the cross-linker was identified as BDDE, which also is in agreement with the information in the product technical files. Five of the investigated products (samples A097401 to A097415) were found to contain lidocaine. Also these findings matched the information in the product leaflets.

4.2 Cross-linking grade of hyaluronic acid-based fillers

To prevent hyaluronic acid from being metabolized too fast after injection, it is often cross-linked. Most of the hyaluronic acid-based fillers in this study were cross-linked and all of these were cross-linked using BDDE. BDDE contains two reactive epoxide groups allowing it to bridge between two strands of hyaluronic acid. Should only one epoxide react with hyaluronic acid, the other epoxide is hydrolysed, yielding a modified hyaluronic acid. The modification grade as well as the cross-linking grade of the hyaluronic acid was determined. The modification grade is defined here as the amount of BDDE relative to the amount of hyaluronic acid. By LC-MS specifically the BDDE bound hyaluronic acid saccharides relative to the total amount of saccharides is determined. By NMR spectroscopy the molar ratio between BDDE and hyaluronic acid monomer is determined. The crosslinking grade is here defined as the percentage of BDDE linked on two hyaluronic acid fragments relative to the total amount of hyaluronic acid fragments. The basic chemical structure of a BDDE-linked hyaluronic acid fragment is shown in Figure 4.2.

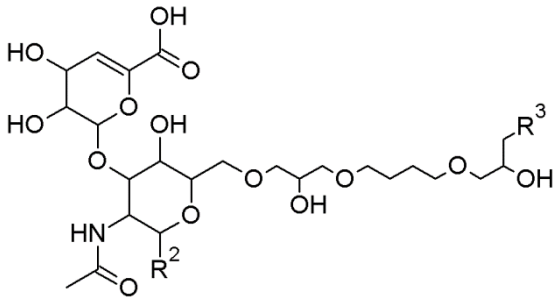

Figure 4.2: Structure of a BDDE modified HA fragment. R^2 and R^3 represent an HA fragment or a terminal alcohol.

Both the modification grade and the cross-linking grade of all hyaluronic acid-based fillers were determined. The results are shown in Figures 4.3 and 4.4 respectively, as well as in Table 4.1.

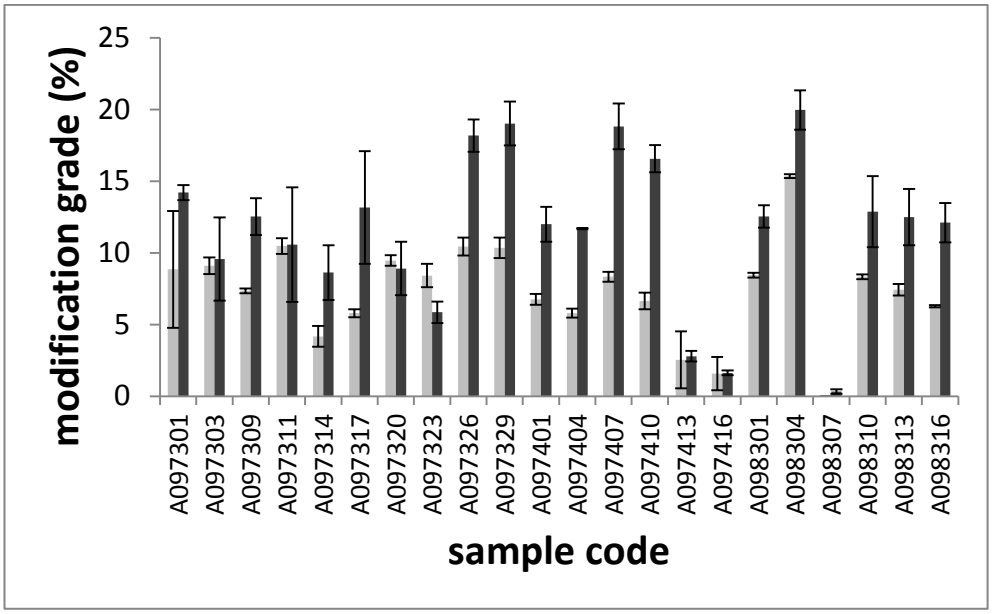

Figure 4.3: Modification grade of the various products as determined by NMR spectroscopy (light grey) and LC-MS (dark grey). Of each type of filler two or three batches were provided which all received an unique A-number (see Annex 7). Data represent the mean of all two or three batches which for clarity in this figure is reflected by just one A-number (sample code). Sample A098307 does not contain a cross-linker, as shown by the presence of only a background signal.

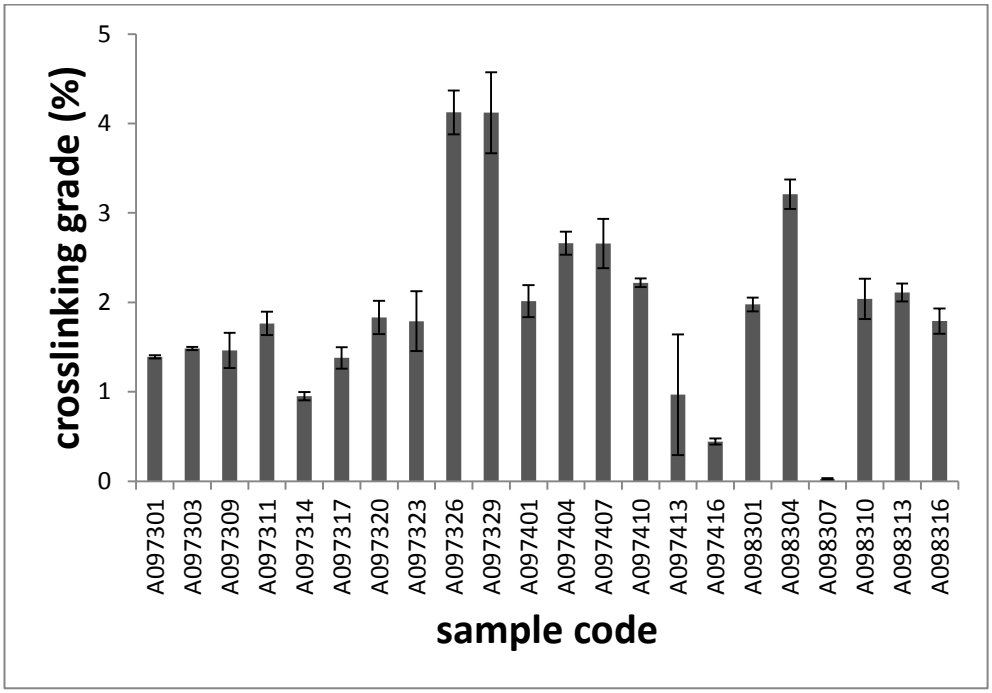

Figure 4.4: Cross-linking grade of the various products as determined by LC-MS. Of each type of filler two or three batches were provided which all received an unique A-number (see Annex 7). Data represent the mean of all two or three batches which for clarity in this figure is reflected by just one A-number (sample code). Sample A098307 does not contain a cross-linker, as shown by the presence of only a background signal.

Table 4.1: Cross-linking grade of the hyaluronic acid-based fillers.

Sample code	Determined cross-linking grade (%)	Declared amount HA in product (%)	Declared amount cross-linked HA in product (%)	Declared BDDE/HA ratio in synthesis (%)
A097301	1.4	2.25	2.25	
A097303	1.5	2.55	2.55	
A097309	1.5		2.0	7
A097311	1.8		2.4	9
A097314	1.0	2	2.0	
A097317	1.4	2.25	2.25	
A097320	1.8		2.5	
A097323	1.8		2.5	
A097326	4.1	2.0	1.92	
A097329	4.1	2.0	1.92	
A097401	2.0	2.4		11*
A097404	2.7	2.0		5.5
A097407	2.7		2.0	
A097410	2.2		2.0	
A097413	1.0		2.0	
A097416	0.4		1.2	
A098301	2.0		2.0	
A098304	3.2		2.5	
A098307	0.0	1.8		0
A098310	2.0		2.3	
A098313	2.1		2.4	
A098316	1.8		1.6	

* 5% additional non-cross-linked HA was added

4.2.1 Comparison to technical files

There are no guidelines on how cross-linking should be defined or determined. The experimentally determined cross-linking grades were compared to what was stated about cross-linking in the product technical files. In most technical files a percentage of cross-linking grade was not reported. Instead, the amount of cross-linked hyaluronic acid was often given. This cross-linked hyaluronic acid could contain any amount of BDDE. In some cases, the amount of BDDE relative to hyaluronic acid used in the production was reported. The reported values as well as the experimental results are shown in Table 4.1. The determined cross-linking grades span a relatively small range from 1.0 to 4.1%, which may be typical for the type of products in the study.

The grade of cross-linking is a parameter independent from the amount of hyaluronic acid present in the product. It is likely that the grade of cross-linking is dependent on the amount of BDDE added to the hyaluronic acid in the production of the filler. This can be seen when looking at the determined cross-linking grade of products A097309 and A097311 and the ratio BDDE/HA used in synthesis. This correlation fails, however, when looking at products A097401 and A097404. Here it could

be that the non-cross-linked hyaluronic acid added to A097401 caused the poor correlation. Nevertheless, less BDDE compared to what was used in producing A097309 and A097311 yields a higher experimental cross-linking grade in A097404. There is no clear trend between the experimental values and what can be found in the technical files. Adding information on the crosslinking grade in the technical files would be useful for authenticity testing of the product and could prevent products like Hyacorp from entering the market.

4.3 Identity of non-hyaluronic acid-based fillers

The identity of the non-hyaluronic acid-based fillers was determined by SEM-EDX. The details of the methods are described in Annex 8. The dermal fillers based on calcium hydroxylapatite and poly-ε-caprolacton were observed to contain spherical particles, whereas the dermal fillers based on poly-L-lactic acid contained amorphous particles, see Figure 4.5.

The molecular formula of calcium hydroxylapatite is $Ca_{10}(PO_4)_6(OH)_2$, and indeed, a high intensity of Ca, P and O was determined in the elemental spectrum of the spheres of this dermal filler, see Table 4.2. There was a high intensity C contribution as well, but this came from the carbon tab background. The molecular formula of poly-ε-caprolacton is $C_6H_{10}O_2$, which is in agreement with the high contribution of C and O found on the spheres of the two dermal fillers of this type. There was no presence of hyaluronic acid in these products, as determined by LC-MS and NMR (data not shown). Na, Cl, S and P were found in the background, which is likely to come from the buffer salts. The molecular formula of poly-L-lactic acid is $C_3H_4O_2$, which is in agreement with the high contribution of C and O found on the spheres of this dermal filler. Na, S and P were found in the background, which likely comes from the buffer salts. No additional particles or elements were found in any of the samples analysed.

Table 4.2: Chemical composition of the non-hyaluronic acid based-filler particles as determined by SEM-EDX on a carbon pad.

Sample code	Declared material	Average elemental composition particles*
A097306	calcium hydroxylapatite $Ca_{10}(PO_4)_6(OH)_2$	P, Ca, O
A097307		P, Ca, O
A097419	poly-ε-caprolacton $C_6H_{10}O_2$	C, O
A097420		C, O
A097422	poly-ε-caprolacton $C_6H_{10}O_2$	C, O
A097423		C, O
A097425	poly-L-lactic acid $C_3H_4O_2$	C, O
A097426		C, O

* The elements are listed in order of the intensity of the signals. Protons cannot be determined.

The average size of the spheres in the calcium hydroxylapatite and poly-ε-caprolacton fillers was determined based on the SEM images.

Representative images obtained using SEM are shown in Figure 4.6. The results of the determination of the sizes are summarized in Table 4.3.

Table 4.3: Particle size of the non-hyaluronic acid-based fillers as determined using their SEM images.

Sample code	No. of particles measured	Determined diameter* (µm)	Declared diameter** (µm)
A097306	157	32 (9 – 45)	25 – 45
A097307	156	32 (5 – 44)	
A097419	80	36 (11 – 55)	25 – 50
A097420	89	35 (16 – 51)	
A097422	59	33 (8 – 59)	25 – 50
A097423	95	37 (19 – 59)	
A097425	No spherical particles visible		d (10) ≥10 µm d (50) 36-60 µm d (90) ≤105 µm
A097426	No spherical particles visible		

* Data represent average diameter plus minimum and maximum range in brackets
** Data taken from the technical files or leaflets

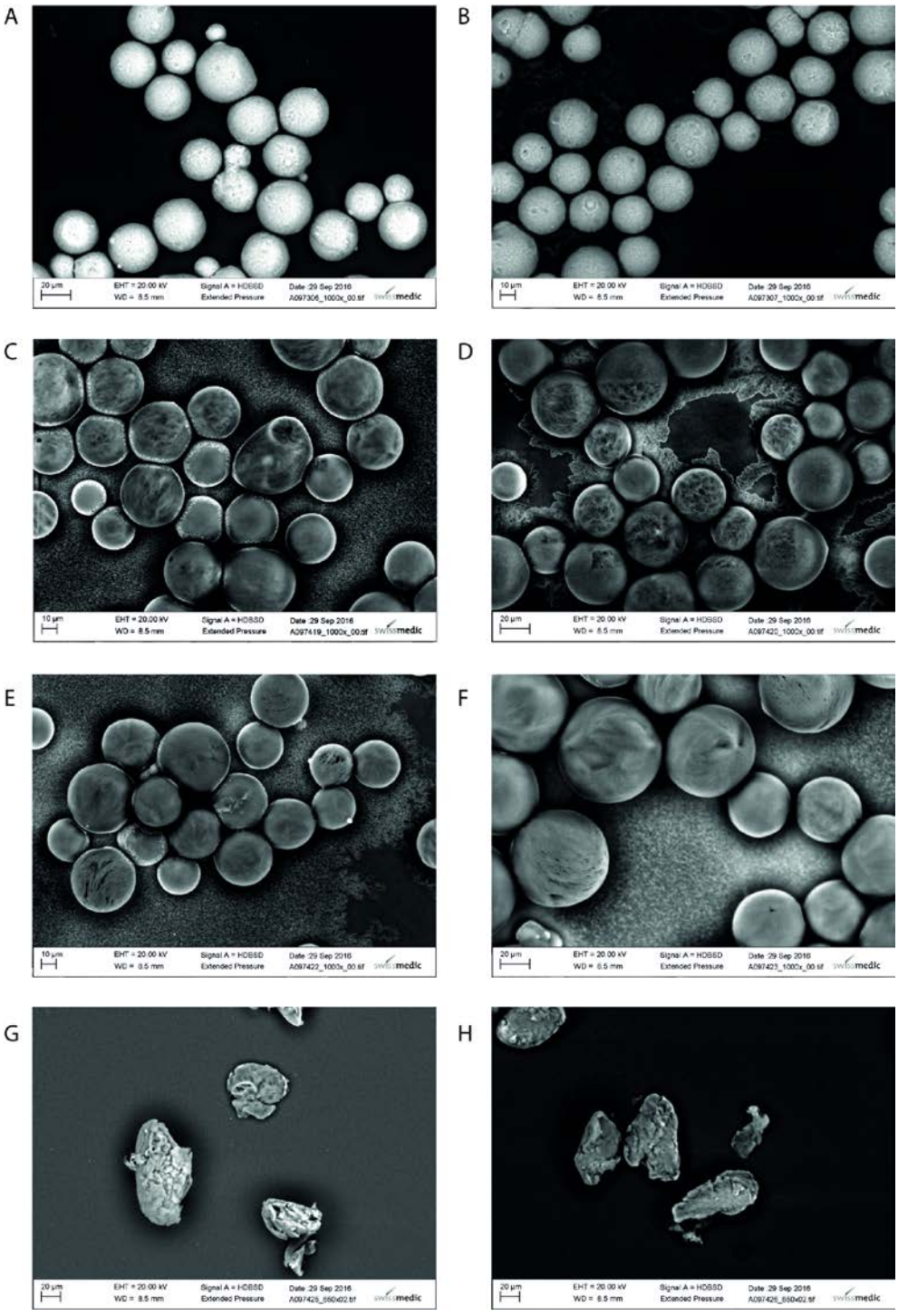

Figure 4.6: SEM images of the non-hyaluronic acid based-fillers. A: order number A097306, B: order number A097307, C: order number A097419, D: order number A097420, E: order number A097422, F: order number A097423, G: order number A097425, H: order number A097426.

4.3.1 Comparison to technical files

The elemental composition of the dermal fillers based on other material than hyaluronic acid matched the information in both the leaflet and the technical file. For all fillers with visible spherical particles, their average dimensions were in agreement with the product specifications. However, the range in particle dimensions exceeded the product specifications for all products. In all cases smaller particles were found and in some cases larger particles as well. The given ranges seem rather small for the total products and are likely to describe the majority of the particles. For products A097425 and A097426, a distribution of particle sizes is given (see Table 4.3). It seems that a similar approach would be a more accurate description for the other products as well. In the description of the size distribution for products A097425 and A097426 after d(10) a smaller or equal (≤) is expected to indicate that 10% of the particles is smaller or equal to 10 µm, rather than a larger or equal sign.

4.4 Discussion and conclusions

Most of the products encountered in the market surveillance study are based on BDDE treated hyaluronic acid. Treatment with BDDE may yield different products depending on the exact manufacturing conditions [23]. We have therefore determined not only the presence of BDDE on the hyaluronic acid chains but also whether this BDDE was a modification or a cross-link. It should be pointed out that there is no internationally accepted definition of modification or cross-linking. Because the manufacturers' interpretation was not found among the received technical documentation, comparison of our results to that of the manufacturers' specifications should be interpreted with caution. To be able to identify and compare products it would be useful if manufacturers could come to generally accepted definitions and harmonised analytical methodologies.

The modification grades determined here by NMR spectroscopy range from 0 to 15%, with an average of 7%. The cross-linking grades determined here by LC-MS range from 0 to 4%, with an average of 2%. These values indicate that the various products are similar regarding to their BDDE treatment and no extremely high values are found. There were no comparable declared cross-linking values in the technical files. As this parameter can influence the filler lifetime and contributes to the fingerprint of the product, it would be useful to have it characterized in the technical files. This information could help in the case of quality issues and counterfeiting. As the market share of fillers is substantial, counterfeiting of the products is likely to happen.

In the market survey, a few non-hyaluronic acid based products were encountered. One of these products is based on a mineral, calcium hydroxylapatite and two others are based on organic polymers, poly-ε-caprolacton and poly-L-lactic acid. The products based on the calcium hydroxylapatite and poly-ε-caprolacton contain spherical particles, the particles based on poly-L-lactic acid have a more amorphous shape. In all cases, micrometer-scale particles were observed using scanning electron microscopy. Only particles in the micrometer range have been investigated in this study. It is not expected that particles from 9 µm or 59 µm (determined values) would have other effects on patient safety

than particles of 25 µm or 50 µm (declared values), as these are still in the same order of magnitude.

In general, the results of all chemical analyses were in agreement with the declared composition of the investigated products. Inconsistencies were not encountered, with one possible exception regarding the particle size of the non-hyaluronic acid-based spherical dermal fillers which seems to exceed the product specifications.

5 Biocompatibility

Biocompatibility assays for the biological evaluation of medical devices comprise a wide range of assays ranging from *in vitro* genotoxicity, haemotoxicity and cytotoxicity assays to *in vivo* sensitization and repeated dose toxicity tests that should be considered according to ISO 10993-1:2009 [21]. One of the most used assays for evaluation of biocompatibility is an *in vitro* cytotoxicity assay using an *in vitro* cell culture system as described in ISO 10993-5:2009 [24]. This assay provides a relatively quick screening to determine potential toxicity or leaching of toxic compounds from a medical device. For a complete evaluation of biocompatibility, a combination of a variety of tests is necessary. However, this is beyond the scope of this study. Therefore, we focus on two types of cytotoxicity assays. The methods used are described in Annex 9. A detailed overview of the results is presented in Annex 10. In total, 26 dermal fillers of 14 different manufacturers were evaluated, which includes two subtypes of fillers per manufacturer, if available.

5.1 Cytotoxic activity

Of the evaluated dermal filler materials none showed cytotoxic activity when extracts were incubated in either RAW264.7 macrophages or L929 fibroblasts. Survival after exposure to the extracts was approximately 90% when compared to the control condition (non-treated cells), see Figures 5.1 and 5.2. Taking into account experimental variability, cytotoxicity is generally considered to occur when cell survival is below 70% of that of the control cells (ISO 10993-5:2009 [23]). The DMSO positive cytotoxic control induced a moderate cytotoxicity with a cell survival of 60% and 50% for RAW264.7 macrophages and L929 fibroblasts, respectively.

Cell membrane integrity, as a second toxicity read out, was not affected by the DMSO positive toxicity control. The results of both RAW264.7 and L929 cells can be found in Figures 5.3 and 5.4, respectively. Membrane integrity after exposure to the extracts was approximately 100% when compared to the control condition (non-treated cells).

Extraction of filler product A098307, which is based on non-cross-linked hyaluronic acid, resulted in a viscous extract that remained after the filtration. To remove the viscous material an additional filtration step was performed after extraction of the filler product. As an extra control, some other cross-linked products were treated similarly. In addition, higher levels of DMSO and Sn-stabilized PVC were tested (see Annex 9). With all of the products a membrane integrity of over 70% was found, whereas in the presence of DMSO and Sn-stabilized PVC the membrane integrity was well below 70% of that of the control condition, see Figures 5.5. and 5.6.

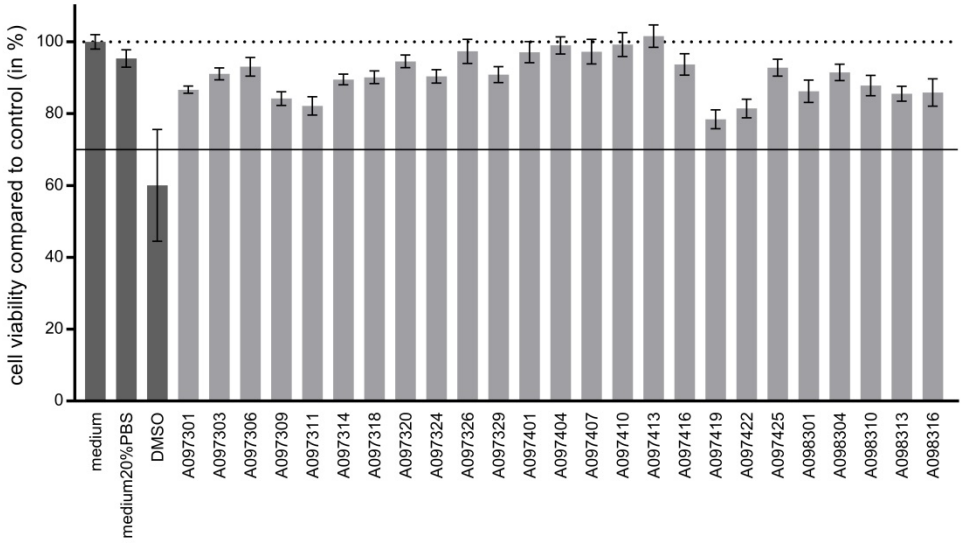

Figure 5.1. Viability of RAW264.7 macrophages after incubation with extracts of dermal filler materials. Of each type of filler two or three batches were provided which all received an unique A-number (see Annex 7). Data represent the mean of two batches (except for product A097301 of which one batch is tested) which for clarity in this figure is reflected by just one A-number. Cytotoxicity is considered to occur when cell viability is below 70% of that of the control condition (medium) and is reflected by the horizontal line.

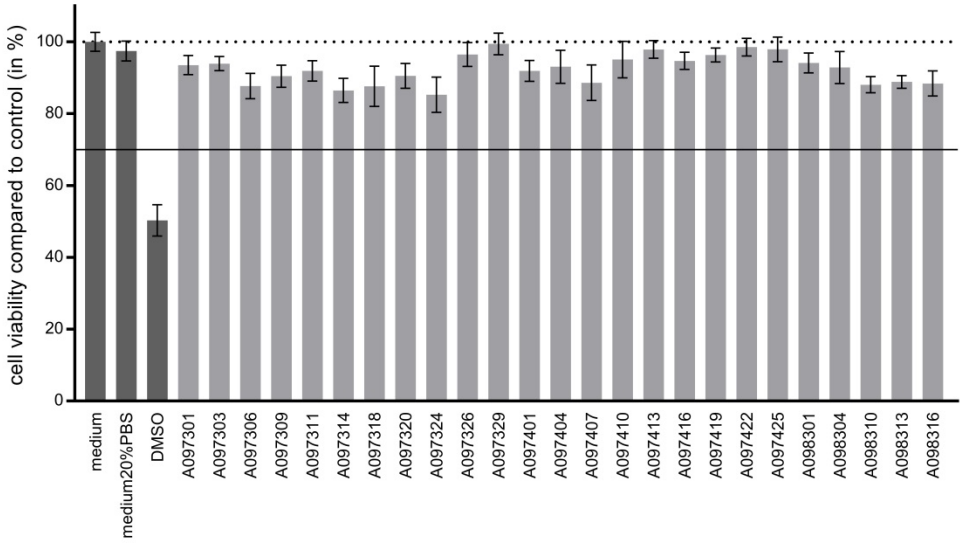

Figure 5.2. Viability of L929 fibroblasts after incubation with extracts of dermal filler materials. Of each type of filler two or three batches were provided which all received an unique A-number (see Annex 7). Data represent the mean of two batches (except for product A097301 of which one batch is tested) which for clarity in this figure is reflected by just one A-number. Cytotoxicity is considered to occur when cell viability is below 70% of that of the control condition (medium) and is reflected by the horizontal line.

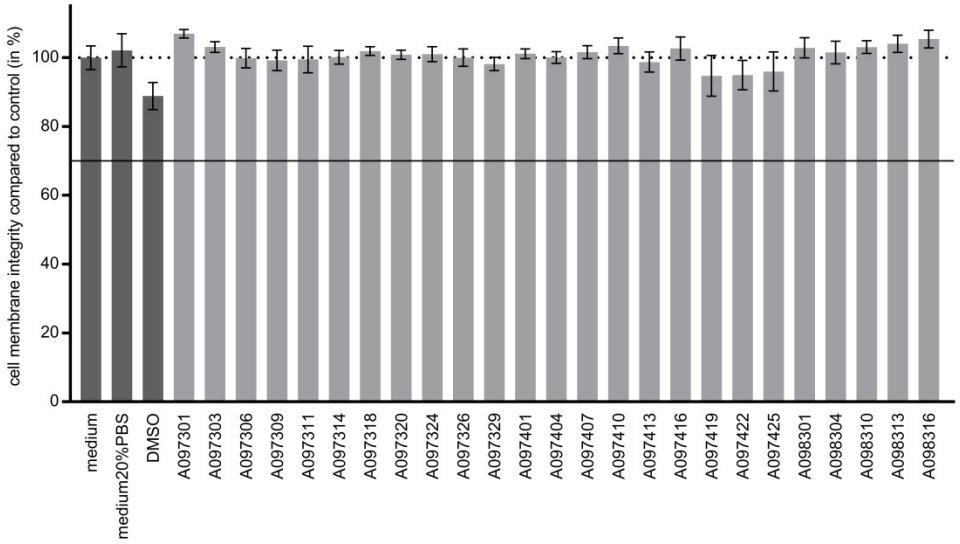

Figure 5.3. Membrane integrity in RAW264.7 macrophages after incubation with extracts of dermal filler materials. Of each type of filler two or three batches were provided which all received an unique A-number (see Annex 7). Data represent the mean of two batches (except for product A097301 of which one batch is tested) which for clarity in this figure is reflected by just one A-number. Cytotoxicity is considered to occur when membrane integrity loss is below 70% of that of the control condition (medium) and is reflected by the horizontal line.

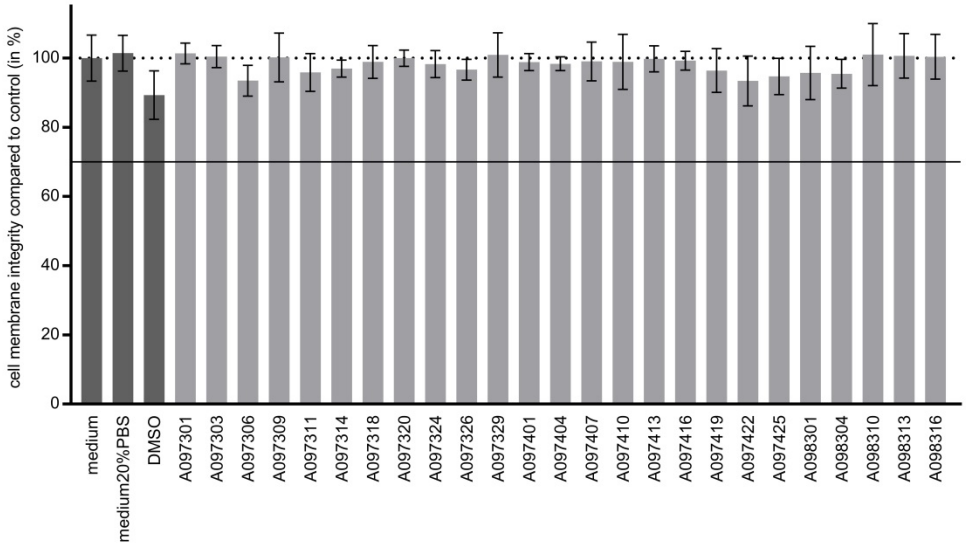

Figure 5.4. Membrane integrity in L929 fibroblasts after incubation with extracts of dermal filler materials. Of each type of filler two or three batches were provided which all received an unique A-number (see Annex 7). Data represent the mean of two batches (except for product A097301 of which one batch is tested) which for clarity in this figure is reflected by just one A-number. Cytotoxicity is considered to occur when membrane integrity loss is below 70% of that of the control condition (medium) and is reflected by the horizontal line.

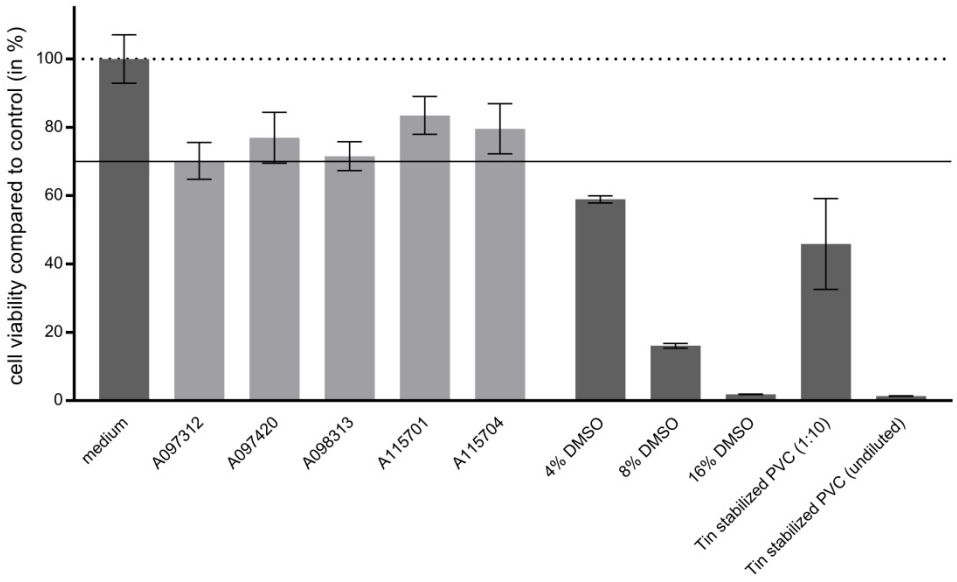

Figure 5.5. Viability in L929 fibroblasts after incubation with extracts of dermal filler materials. Two types of filler products (A098307 and A098310) were newly provided for this alternative experiment and as such have new A-numbers (see Annex 9). Of these products, three batches were tested, which for clarity in the figure is reflected by just one A-number. Of the controls (the previously tested products A09712, A097420 and A098313) only one batch was tested. Cytotoxicity is considered to occur when cell viability is below 70% of that of the control condition (medium) and is reflected by the horizontal line.

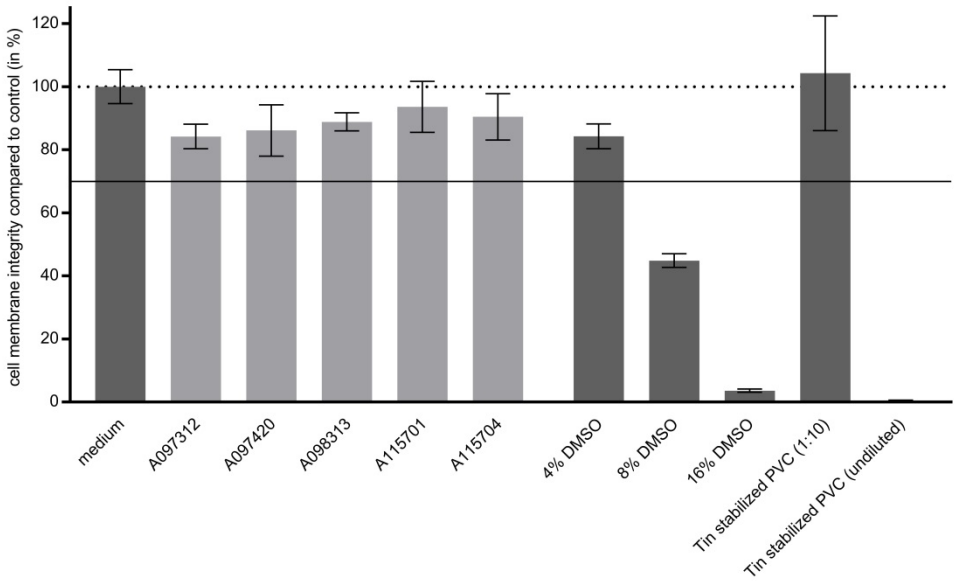

Figure 5.6. Membrane integrity in L929 fibroblasts after incubation with extracts of dermal filler materials Two types of filler products (A098307 and A098310) were newly provided for this alternative experiment and as such have new A-numbers (see Annex 9). Of these products, three batches were tested, which for clarity in the figure is reflected by just one A-number. Of the controls (the previously tested products A09712, A097420 and A098313) only one batch was tested. Cytotoxicity is considered to occur when membrane integrity loss is

below 70% of that of the control condition (medium) and is reflected by the horizontal line.

5.2 Discussion and conclusions

To summarize, it can be concluded that for 26 different dermal filler materials of 14 different manufacturers no significant cytotoxicity was observed, showing that the dermal filler materials used for a broad range of filler brands are non-toxic for cells. These results are an indication of biocompatibility of the studied dermal filler materials. However, it should be noted that for a full biocompatibility evaluation also other studies according to ISO 10993-1 should be considered and performed.

6 Overall discussion and conclusions

This study addresses non-permanent dermal fillers available on the Dutch market. Non-permanent dermal fillers were selected based on a combination of a scientific literature search and a questionnaire performed amongst appliers of dermal fillers. Technical documentation provided by manufacturers as well as experimental data on physicochemical characteristics and biocompatibility were obtained to study the selected non-permanent dermal fillers. Twenty-six non-permanent dermal fillers from 14 different manufacturers were identified to be available on the Dutch market in 2014. Several key physicochemical and biocompatibility characteristics of the products as determined in the laboratory analysis were good. The technical documentation of most products contained shortcomings at some aspects. Complete and correct documentation is the basis to warrant patient safety. Although the potential impact on patient safety of the particular shortcomings found in the files is expected to be limited, they should be carefully considered and resolved by the manufacturers in order to substantiate the quality and safety of their products as required in the regulatory system. To arrive at this over-all conclusion, five questions were addressed as described below.

Which non-permanent dermal fillers are being used in the Netherlands?
Fourteen non-permanent dermal filler brands were identified to be available on the Dutch market. Most non-permanent dermal fillers consist of hyaluronic acid, but also fillers based on calcium hydroxylapatite, poly-L-lactic acid and poly-ε-caprolacton are being used. In our survey, a total of 17,169 treatments with non-permanent dermal fillers was reported. Only a few adverse reactions were reported ranging from no effect and temporary swelling to allergic reactions and infections. No severe adverse reactions were reported. The limited number of records may either indicate that adverse reactions do rarely occur or that adverse reactions are underreported.

Do the technical files of the selected non-permanent dermal fillers provide adequate proof of conformity with the requirements of the Medical Devices Directive (MDD)?
The assessment score varied considerably per dermal filler documentation set. One technical file did not show any shortcomings, in all others one or more shortcomings were found. In general, the score on the item Clinical evaluation was poor, often due to an incomplete equivalence claim. However, shortcomings in the technical file do not necessarily mean that the device is of insufficient quality.

Are key physicochemical characteristics of the products, such as material identity and degree of cross-linking, in line with the information in the technical documentation?
Most of the products encountered in the market surveillance study were shown to be based on BDDE treated hyaluronic acid, which matched the information in the technical documentation. The determined cross-linking grade of the hyaluronic acid ranged from 0 – 4%, with an average of 2%. These values could not directly be compared to a

declared cross-linking grade, as such data was not provided in the technical documentation. There is no internationally accepted definition of cross-linking, let alone an accepted analytical method to determine it. As a high cross-linking grade was suggested to be the reason for the side effects caused by the fillers from Hyacorp, it should be encouraged to specify this parameter for filler products in the technical files. A consensus on definitions and accepted analytical methods would be a first logical step.

The chemical composition of the dermal fillers based on other material than hyaluronic acid matched the information in both the leaflet and the technical file. Inconsistencies were not encountered with one possible exception regarding the particle size of a non-hyaluronic acid-based spherical dermal filler, which seems to exceed the specified range. The specified range did match with the determined average size but did not cover the full size distribution. This is not expected to compromise patient safety however.

In general, the results of all chemical analyses were in agreement with the declared composition of the investigated products.

Is the material as present in the products free of cytotoxic activity, as part of screening for biocompatibility?
Cytotoxic activity was determined using two different *in vitro* assays performed in two different cell lines. In 26 different dermal filler materials obtained from 14 different manufacturers, no significant cytotoxicity was observed. Therefore, it is concluded that the dermal filler materials used for a broad range of filler brands, are non-toxic for cells and as such indicate good biocompatibility. However, it should be noted that for a full biocompatibility evaluation also other studies according to ISO 10993-1 should be considered and performed.

In case of shortcomings, do these lead to a concern for patient safety?
The regulatory system of medical devices depends to a large extent on the quality of the submitted technical documentation. Therefore, any shortcomings in that documentation could imply that product safety and safe use of the device are insufficiently guaranteed. However, shortcomings in a technical file do not necessarily mean that the device is of insufficient quality. An analysis of the shortcomings showed that of most shortcomings the potential impact on patient safety can be considered limited since these have a more administrative character. However, in one technical file, the PMS data analysis was insufficiently documented and in another file, the item ‘risk analysis’ showed shortcomings. In these two cases, product safety cannot sufficiently be guaranteed. Shortcomings in the technical files should be carefully considered and resolved by the manufacturers in order to substantiate the quality and safety of their products as required in the regulatory system.

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Inspectie voor de Gezondheidszorg
Ministerie van Volksgezondheid, Welzijn en Sport

Annex 1: Request of IGZ

> Retouradres Postbus 2680 3500 GR Utrecht

Stadsplateau 1
3521 AZ Utrecht
Postbus 2680
3500 GR Utrecht
T 088 120 50 00
F 08 120 50 0
www.igz.nl
meldpunt@igz.nl

Ons kenmerk

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Datum 8 oktober 2015
Onderwerp Enquête t.b.v. onderzoek naar non-permanente fillers

Geachte heer / mevrouw,

Het Rijksinstituut voor Volksgezondheid en Milieu (RIVM) vraagt uw hulp bij het verzamelen van informatie over fillers. Het RIVM doet in opdracht van de Inspectie voor de Gezondheidszorg (de inspectie) onderzoek naar karakteristieke eigenschappen van rimpelvullers (fillers) die worden toegepast in Nederland. Op grond van deze gegevens kunnen onder andere toezichtprioriteiten worden gesteld t.a.v. van producten die in meer of mindere mate risicovol kunnen zijn.

Wat is het doel van het RIVM en de inspectie?
Het RIVM wil in opdracht van de inspectie onderzoek doen naar chemische eigenschappen van fillers (bijvoorbeeld: crosslinking, concentratie hyaluronzuur, etcetera). Het doel bij dit onderzoek is kijken of er eigenschappen te identificeren zijn die van invloed zijn op de reactie van het lichaam op de filler. Om dit onderzoek goed uit te voeren, wil het RIVM een aantal producten die tot relatief weinig klachten leiden vergelijken met een aantal producten die tot relatief veel klachten leiden. De inspectie heeft geen goed beeld van de producten die aan beide beschrijvingen zouden kunnen voldoen. Met behulp van deze enquête willen het RIVM en de inspectie zicht te krijgen op de fillers die op de Nederlandse markt zijn, hoe vaak die ten opzichte van elkaar ongeveer toegepast worden en met welke fillers goede en slechte ervaringen zijn opgedaan.

Waarom ontvangt u deze e-mail?
Het RIVM en de inspectie besloten voor dit onderzoek alle leden van de NSEG aan te schrijven. Als lid van een wetenschappelijke vereniging die is aangesloten bij de NSEG ontvangt u deze vragenlijst.

Waarom ontvangt u deze e-mail op dit moment?
Mede door politieke en maatschappelijke aandacht voor de cosmetische sector heeft de inspectie sinds enkele jaren meer capaciteit ingezet voor het toezicht op – onder meer - de fillers. Recente incidenten met een enkele filler op de Nederlandse markt en de toename van het aantal beschikbare

fillers, riepen bij de inspectie de vraag op of specifieke eigenschappen van fillers op voorhand een indicatie kunnen zijn voor het voordoen van incidenten. Vanuit deze vraag is het huidige onderzoek voortgekomen. Dit onderzoek staat los van andere ontwikkelingen in de sector of onderzoeken van de inspectie of het RIVM.

Welke informatie uit uw reactie wordt met de inspectie gedeeld?
Wanneer u de enquête invult en retourneert, zal het RIVM de informatie verwerken en anonimiseren. De gegevens die het RIVM met de inspectie deelt en/of gepubliceerd worden zullen niet tot uw persoon of uw instelling terug te herleiden zijn. De focus van het onderzoek ligt uitsluitend op de gebruikte producten, niet op de gebruiker.

Wat kan het belang van dit onderzoek zijn voor u?
Het RIVM hoopt eigenschappen te kunnen identificeren die mogelijk risicofactoren zijn voor het ontstaan van klachten. Tevens worden de uitkomsten vergeleken met de technisch dossiers van de fabrikant. De uitkomsten kunnen de inspectie helpen bij het stellen van prioriteiten bij het toezicht op deze producten. Het RIVM en de inspectie zijn voornemens de uitkomsten - waar mogelijk – terug geven aan het veld. Gebruikers kunnen hier mogelijk hun voordeel mee doen bij de keuze voor de producten die zij gebruiken.

Hoeveel tijd kost deze enquête?
Het RIVM en de inspectie vragen een wat hogere mate van detaillering met betrekking tot de producten die u toepast. Merken brengen doorgaans series van producten met verschillende eigenschappen op de markt. Voor het onderzoek naar de specifieke eigenschappen is het belangrijk zo precies mogelijk te weten om welk product uit de serie (welk type) het gaat. Het vraagt mogelijk wat van uw tijd om na te gaan welke verschillende typen producten u gebruikt(e) of waar u in het recente verleden goede of minder goede ervaringen mee had. Voor wat betreft het aantal behandelingen en klachten volstaan zo goed mogelijke schattingen.

Wanneer en hoe kan ik reageren op deze enquête?
Indien er in uw praktijk/instelling wel non-permanente fillers toe worden gepast voor cosmetische doeleinden, maar u de vragen zelf niet voldoende kunt beantwoorden, verzoek ik u deze brief door te sturen naar een collega in uw praktijk die dat wel zou kunnen. Indien er door u en in uw praktijk helemaal geen non-permanente fillers voor cosmetische doeleinden worden toegepast, hoeft u niet te reageren.

De inspectie verzoekt u vriendelijk de vragenlijst in de bijlage in te vullen en aan het RIVM te retourneren. U kunt het formulier per e-mail naar het volgende adres sturen: **fillers@rivm.nl**. Ook eventuele vragen over deze enquete kunt u via dit e-mailadres stellen.

Graag ontvangt het RIVM de ingevulde vragenlijst **binnen drie weken na ontvangst van dit bericht** retour. Uw medewerking aan het onderzoek wordt zeer gewaardeerd.

Inspectie voor de Gezondheidszorg
Ministerie van Volksgezondheid,
Welzijn en Sport

Met vriendelijke groet,

Senior inspecteur - coördinator van dit onderzoek namens de inspectie
Afdeling medische technologie
Inspectie voor de Gezondheidszorg

Stadsplateau 1
3521 AZ Utrecht
Postbus 2680
3500 GR Utrecht
T 088 120 50 00
F 08 120 50 0
www.igz.nl
meldpunt@igz.nl

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BIJLAGE 1: Vragenlijst voor gebruikers van non-permanente fillers

Ten behoeve van een onderzoek uit te voeren door het RIVM wil de IGZ u vragen onderstaande vragen te beantwoorden in de bijbehorende tabel.

1. Welke non-permanente fillers past u toe voor cosmetische doeleinden? Graag de merknaam en de volledige omschrijving van serie/type en nummer indien van toepassing.

2. Beschrijf het hoofdbestanddeel of de werkende stof (bijvoorbeeld: hyaluronzuur, polymelkzuur, etcetera).

3. Wie is uw leverancier voor deze producten (niet noodzakelijkerwijs de fabrikant van het product)? Indien mogelijk ook de websites noemen: dit kan de inspectie helpen bij het opvragen van technische dossier bij de leverancier / fabrikant.

4. Hoeveel behandelingen heeft u met elk van de genoemde producten ongeveer uitgevoerd in 2014-2015? Een schatting is voldoende.

5. Welke producten hebben in de laatste 3 jaar geleid tot klachten? Indien u klachten of complicaties bekend zijn, deze graag in de laatste kolom categorisch specificeren met een schatting van het aantal keren dat ze voorkwamen.

Merknaam + type + no. (indien van toepassing)	**Hoofdbestand deel of werkende stof**	**Leverancier (en websites)**	**Aantal behandelingen 2014**	**Klachten + aantal**
Voorbeeld: Fictieffill Ultra 2 *	hyaluronzuur	Fictief Esthethiek (fictief.nl)	500	Verharding 8 Ontsteking 3 Kapselvorming 2

Annex 2: Methods of assessment of technical documentation

6.1.1 Technical documentation requested

The Dutch Health Care Inspectorate (IGZ, since 1 October 2017 'IGJ') requested a relevant part of the technical documentation of the selected dermal fillers from the accompanying manufacturers (see Annex 3 for a copy of the letter), in order to process the information and report on it anonymously in an RIVM letter report. With the letter requesting the technical file, a checklist was enclosed which described details on the items to be submitted (see Annex 4). The checklist was developed by RIVM and was largely based on the Summary Technical Documentation (STED) from the Global Harmonisation Task Force[1] [25]. Importantly, the information to be requested, was selected based on the relationship of the information with the risks associated with dermal fillers or their safe use. The following information was requested from the manufacturers:

1. Device description
2. Instructions for use
3. Risk analysis
4. Product verification and validation – relevant parts for this investigation:
   - General
   - Biocompatibility testing
   - Physical testing
   - Clinical evaluation
5. Summary and analysis of post-market surveillance (PMS) data.

Following receipt, the documentation was checked for completeness and any missing documentation was requested once more.

Assessment method

An assessment form (see Annex 5) was developed in order to enable a structured and uniform assessment of the documentation sets. For each section of the checklist from Annex 4, a file item was included and for each item a set of sub-items was listed (largely based on the additional information listed in the STED).

In general, the assessment was based on the presence/description of each particular sub-item in the documentation. For most sub-items, presence of adequate information was scored with 'no', 'partially', or 'yes', if applicable. For the summary of Post Market Surveillance (PMS) data and conclusion, a dedicated terminology was used ('no', 'limited', 'clear').

[1] The Global Harmonization Task Force (GHTF) was the predecessor of the current International Medical Device Regulators Forum (IMDRF). IMDRF aims to accelerate international medical device regulatory harmonization and convergence. GHTF final documents are still current and can be accessed on the IMDRF website. As the work of IMDRF progresses, these documents will be reviewed and published as IMDRF documents. For more information, see http://www.imdrf.org/index.asp.

No score was assigned to device description, which was only used as background information for the assessment. For the instructions for use (IFU), it was checked whether specific dermal filler-related risks (see Annex 5, Attachment I) were mentioned. For the assessment of the risk analysis, it was checked whether these dermal filler-related risks were addressed as well as whether general hazard categories (see Annex 5, Attachment II), as derived from the harmonized standard for risk management of medical devices, were covered [20]. For the clinical evaluation, a list of dermal filler-related topics to be covered was drawn up and checked (see Annex 5, Attachment III). Using expert judgment of the RIVM, a higher weight and, consequently, a higher score was given to important sub-items related to risk and safety aspects. Therefore, the scoring system discriminates sub-items of normal importance and sub-items of major importance (see also Annex 5). To provide the possibility to comment on assigned scores and to include additional findings in the assessment form, an option was created to give qualifying remarks for every item. These remarks can be used in the discussion of the results.

To facilitate consistent assessment, the documentation was assessed independently by two assessors. Assessment forms were compared and any discrepancies were discussed and resolved. This method has also been used for previous investigations on metal-on-metal hip implants [26], silicone breast implants [18], and blood glucose meters [19].

Quality of technical documentation items

The overall score for technical documentation items was obtained as the sum of the sub-item scores. The sum translated into a ‘good’, ‘moderate’ or ‘insufficient’ score. Items scored ‘good’ if the sum was maximal, i.e. every sub-item was adequately addressed and received the maximum of four points for a major sub-item or the maximum of two points for other sub-items. Items scored ‘insufficient’ if one major sub-item (or more) was missing or if a combination of missing or partially addressed sub-items resulted in an equivalent number of missing points (i.e., ≥4), irrespective of the total number of sub-items. For the summary and analysis of PMS data, all sub-items were considered essential and thus, the score of this documentation item was insufficient already when one sub-item was missing (i.e., ≥2 points missing).

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Annex 3: Request of IGZ

> Retouradres Postbus 2680 3500 GR Utrecht

Stadsplateau 1
3521 AZ Utrecht
Postbus 2680
3500 GR Utrecht
T 088 120 50 00
F 08 120 50 0
www.igz.nl
meldpunt@igz.nl

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Dear Sir/Madam,

The Dutch Health Care Inspectorate (Inspectorate) is the competent authority for the European Directive on Medical Devices 93/42/EEC in the Netherlands. As such the Inspectorate is charged with the surveillance and law enforcement of this Directive.

According to the information known to the Inspectorate your company markets dermal fillers in the Netherlands. By request of the Inspectorate, the National Institute for Public Health and Environment (RIVM) will perform a study and laboratory analysis on dermal fillers. Therefore we request you to provide the following information to the Inspectorate:

- Within 1 week after receipt of this letter: the contact details (including name, e-mail address and telephone number) of the person who will be in charge of handling our request on behalf of your company. Additionally, please include the product names / types of the marketed dermal fillers and distributors in/for the Netherlands. These data can be sent by e-mail to _DienstpostbusIGZMedischetechnologie@igz.nl;
- The requested documentation as specified in the attached list. Please, provide the documentation in such a format that it clearly refers to the items as listed in the attachment, in order to prevent misinterpretation during assessment;
- Samples of 3 different batch numbers of the dermal filler products that are marketed by your company in the Netherlands.

You are requested to send the dermal fillers and documentation, marked as confidential to:

The Dutch Health Care Inspectorate
Secretariat Medical Technology
PO Box 2680
3500 GR Utrecht
The Netherlands

If you prefer to submit the documentation electronically, you can send it to:
_DienstpostbusIGZMedischetechnologie@igz.nl

It would be very much appreciated it if you could forward your information before:
March 1, 2016

Please note that additional documentation may be requested, if information is considered to be incomplete or assessment of provided information indicates a need for more information.

Upon finalizing the investigation, I will inform you regarding the findings concerning your dermal filler. If you have any questions regarding this letter or study, please do not hesitate to contact me at the letter head address or at: _DienstpostbusIGZMedischetechnologie@igz.nl

Yours sincerely,

Senior Inspector Medical Technology

Enclosure(s): Documentation required

Annex 4: Checklist for request Dutch fillers

1. Chemical composition / product specifications

- Identity of raw materials (including chemical name);
- Chemical specification of raw materials;
- List of suppliers of raw materials;
- Preparation protocol of the filler;
- Chemical specifications of the filler*;
- Medicinal substance, if applicable.

* Including underlying documentation on requirements of the filler and methods of analysis

2. Device description

- General description, including the indications and contraindications for use, eligibility of persons for treatment, intended anatomical locations/conditions to be treated, and performance (duration of effect);
- An explanation of any novel features.

3. Instructions for use

- The instructions for use of the device as described in essential requirement 13, including requirements 7.5 and 9.1 of the European MDD[1].

[1] For the purpose of the investigation, the instructions for use should be the version associated with the medical device as marketed in the Netherlands.

4. Risk analysis

This documentation should contain a full report (NOT a summary) of the risks identified during the risk analysis process and how these risks have been controlled to an acceptable level. Preferably, this risk analysis should be based on recognised standards, be consistent with the manufacturer's risk management plan, and be in English. The documentation should include:

- The risk management plan;
- Date/version number of risk analysis;
- Reference to any standards used, e.g. EN ISO 14971;
- All hazard categories (for example: Table Annex E of the current standard EN ISO 14971) identified or, appropriately, declared not applicable;
- Estimates of associated risk;
- Risk control, i.e. control measures that are consistently described in line with essential requirement 2 of the European MDD;
- (overall) Justification/acceptability of residual risks in relation to anticipated benefits.

5. Product verification and validation – relevant parts for this investigation;

5.1. General

The documentation should summarise the results of verification and validation studies undertaken to demonstrate conformity of the device

with the essential requirements that apply to it. For this investigation, the information should cover only the following items:

- Biocompatibility (see 5.2);
- Physical testing (see 5.3);
- Clinical evaluation (see 5.4);
- Any literature review performed to support one or more of the above items or the risk analysis should be included.

5.2. Biocompatibility

Detailed information should be included on:

- The tests conducted and reference to standards applied (if applicable);
- Protocols of the in vitro and in vivo studies conducted;
- Summary of results;
- Conclusions.

5.3. Physical testing (e.g. elasticity, viscosity)

Detailed information should be included on:

- The tests conducted;
- Summary of results;
- Conclusions.

5.4. Clinical evaluation

The clinical evaluation report contains the following elements:

- The proprietary name of the medical device and any code names assigned during device development;
- Identification of the manufacturer of the medical device;
- Description of the medical device and its intended application;
- Intended indications;
- Safety and performance claims made for the medical device;
- Context of the evaluation;
- Choice of clinical data types;
- Description of clinical follow-up;
- Summary of the clinical data and appraisal;
- Performance analysis of the medical device;
- Safety analysis of the medical device, including serious adverse events that occurred;
- Consistency of medical device literature and instructions for use with clinical data;
- Conclusions.

More information on the contents of the clinical evaluation report can be found on the website of the International Medical Device Regulators Forum (www.imdrf.org).

6. Summary and analysis of PMS data

The submitted documentation should contain PMS reports of the last four years containing the following elements:

- Summary of PMS data, including specification of the frequency of separate adverse events, complaints, side effects, complications, and description of other experiences related to the use of the product.
- Sources used;
- Analysis of PMS data.

Annex 5: Assessment form

	Medical device code:			
	Notified body (code and name):			
		Options	**Score options**	**Score**
	1. Device description			
1.1	General description, including intended use/purpose	No, yes		
1.2	Intended patient population (eligibility of persons for treatment; indications; conditions to be treated)	No, yes		
1.3	Contraindications	No, yes		
1.4	Intended anatomical locations	No, yes		
1.5	Performance (duration of effect)	No, yes		
1.6	Explanation of novel features	No, yes/NA		
	Qualifying remarks			

	2. IFU			
2.1	Indications for use mentioned	No, yes	0, 2	
2.2	Important aspects of the injection technique	No, partially, yes	0, 2, 4	
2.3	Contraindications and filler-related risk topics mentioned (attachment I)	No, partially, yes	0, 2, 4	
2.4	IFU in Dutch or English	No, yes	0, 2	
	Total		*12*	
		Good	*12*	
		Moderate	*10*	
		Insufficient	*≤8*	
	Qualifying remarks			

	3. Risk analysis			
3.1	Risk management plan	No, yes	0, 2	
3.2	Dated/version number risk analysis	No, yes	0, 2	
3.3	Contraindications and filler-related risk topics addressed (attachment I)	No, partially, yes	0, 2, 4	
3.4	All risk categories addressed (attachment II)	No, partially, yes	0, 2, 4	
3.5	Risks estimated	No, yes	0, 2	
3.6	Risk control/mitigation adequately described	No, partially, yes	0, 2, 4	
3.7	Acceptability of residual risks addressed	No, yes	0, 2	
3.8	Conclusions	No, yes	0, 2	
	Total		*22*	
		Good	*22*	
		Moderate	*20*	
		Insufficient	*≤18*	
	Qualifying remarks			

	4. Biocompatibility			
4.1	Literature review for the biocompatibility investigations	No, partially, yes	0, 1, 2	
4.2	Tests conducted	No, yes	0, 2	
4.3	Appropriateness of the tests conducted	No, partially, yes	0, 1, 2	
4.4	Standards applied	No, yes	0, 2	
4.5	Test protocols	No, partially, yes	0, 1, 2	
4.6	Summary of results	No, yes	0, 2	
4.7	Conclusions	No, yes	0, 2	
	Total		*14*	
		Good	*14*	
		Moderate	*11-13*	
		Insufficient	*≤10*	
	Qualifying remarks			

	5. Physical testing (e.g. elasticity, viscosity)			
5.1	Tests conducted	No, yes	0, 2	
5.2	Appropriateness of the tests conducted	No, partially, yes	0, 1, 2	
5.3	Summary of results	No, yes	0, 2	
5.4	Conclusions	No, yes	0, 2	
	Total		*8*	
		Good	*8*	
		Moderate	*5-7*	
		Insufficient	*≤4*	
	Qualifying remarks			

	6. Clinical evaluation			
6.1	If clinical evaluation report is based on equivalence, is a rationale given to substantiate the equivalence	No, partially, yes/NA	0, 1, 2	
6.2	Proprietary name of the medical device or any code names assigned during device development (if no, the assessment can be stopped)	No, yes	0, 2	
6.3	Identification of the manufacturer of the medical device	No, yes	0, 2	
6.4	Description of the medical device	No, yes	0, 2	
6.5	Intended indications	No, partially, yes	0, 1, 2	
6.6	Safety and performance claims	No, partially, yes	0, 1, 2	
6.7	Objective of the evaluation	No, yes	0, 2	
6.8	Choice of clinical data types (literature, clinical investigation or combination) substantiated	No, yes	0, 2	
6.9	Systematic, documented and appropriate literature search strategy (if applicable)	No, partially, yes/NA	0, 1, 2	
6.10	Description of clinical follow-up	No, yes	0, 2	

6.11	Performance analysis	No, partially, yes	0, 2, 4	
6.12	Safety analysis (incl. evaluation of serious adverse events if clinical investigation is conducted)	No, partially, yes	0, 2, 4	
6.13	Relevant topics adequately addressed (attachment III)	No, partially, yes	0, 2, 4	
6.14	Summary of the clinical data and appraisal (considerations leading to conclusions)	No, partially, yes (both)	0, 2, 4	
6.15	Conclusions	No, yes	0, 2	
	Total		*38*	
		Good	*38*	
		Moderate	*35-37*	
		Insufficient	*≤34*	
	Qualifying remarks			

	7. Summary and analysis of PMS data			
7.1	PMS sources identified	No, yes	0, 2	
7.2	Analysis of PMS data, including frequencies/ratios of adverse events, complaints, side effects, complications, and description of other experiences	No, yes	0, 2	
7.3	Summary of PMS data and conclusions	No, limited, clear	0, 1, 2	
7.4	Actions taken based on the analysis of PMS data	No, yes	0, 2	
	Total		*8*	
		Good	*8*	
		Moderate	*7*	
		Insufficient	*≤6*	
	Qualifying remarks			

Grey-shaded sub-items represent major sub-items

Attachment I: Risks and contraindications based on literature for dermal fillers

It should be checked whether the headings given are addressed, not whether all items are addressed. Tick boxes: first column for instructions for use (IFU) and second column for risk analysis (RA).

IFU	RA	
☐	☐	**1. Contraindications**
☐	☐	Active skin infection, e.g. impetigo, herpes simplex, massive demodex folliculorum, pityrosporum, *Propionibacterium acnes*, viral warts
☐	☐	Active localized infection, e.g. ear, nose, or throat infections, dental abscess, periodontitis
☐	☐	Active generalized infection/systemic infection, e.g. gastroenteritis, urinary bladder infection, tuberculosis
☐	☐	Active inflammatory condition, e.g. pimples, rashes, hives (urticaria)
☐	☐	Active collagenosis, e.g. mixed connective tissue disease, active morphea, active systemic lupus
☐	☐	Allergy/hypersensitivity to the filler material
☐	☐	Allergy to the medicinal substance mixed with the filler, e.g. lidocaine
☐	☐	Allergy manifested by history of anaphylaxis
☐	☐	Glabellar region
☐	☐	Pregnancy
☐	☐	Breast feeding
☐	☐	Bleeding/blood disorder, e.g. haemophilia, haemoglobin pathology, thalassemia
☐	☐	Active anticoagulant medication, e.g. warfarin, clopidogrel, aspirin
☐	☐	High dose of vitamin C
☐	☐	High dose of omega 3 oils
☐	☐	Breast augmentation
☐	☐	Implantation in anatomical areas other than the dermis
☐	☐	Implantation in or close to areas previously treated with a permanent or non-hyaluronic acid dermal filler
☐	☐	Implantation into the periorbital area
☐	☐	(Sub)mucosal implantation for lip augmentation
☐	☐	Hypertrophic or keloid scar formation
☐	☐	Psychiatric diseases or body dysmorphic disorder
☐	☐	Age (younger than 18 years)

Remark:

IFU	RA	
☐	☐	**2. Complications/side effects**
☐	☐	Hematoma (bruise)
☐	☐	Ecchymosis
☐	☐	Pruritus (itch)
☐	☐	Pain
☐	☐	Rash
☐	☐	Erythema (redness)
☐	☐	Oedema (swelling)
☐	☐	Firmness
☐	☐	Tenderness

☐ ☐ Hypoesthesia (numbness)
☐ ☐ Sore (ulcer)
☐ ☐ Discolouration
☐ ☐ Allergic reaction/hypersensitivity/inflammation
☐ ☐ Infection
☐ ☐ Formation of cysts, nodules, granulomas, lumps
☐ ☐ Contour irregularities
☐ ☐ Asymmetries
☐ ☐ Necrosis
☐ ☐ Severe allergic reaction (anaphylactic shock)
☐ ☐ Migration/movement of the filler material from the site of injection
☐ ☐ Leakage of the filler material at the injection site or through the skin
☐ ☐ Vision abnormalities, including blindness
☐ ☐ Stroke
☐ ☐ Injury to the blood supply
☐ ☐ Keloid
☐ ☐ Paresthesia
Remark:

IFU RA
☐ ☐ **3. Injection requirements**
☐ ☐ Physician's experience (authorized medical practitioner, plastic surgeon, dermatologist, specific training)
☐ ☐ Injection technique(s)
Remark:

IFU RA
☐ ☐ **4. Risk factors**
☐ ☐ Amount of filler injected (over a particular period, e.g. one year)
☐ ☐ Mixing with other products before injection
☐ ☐ Handling and disposal of syringes and needles after use
☐ ☐ Exposure of treated areas to excessive sun, UV lamp exposure and extreme hot or cold weather (for the first 24-48 hours after treatment)
☐ ☐ Strenuous exercise (within 24 hours after treatment)
☐ ☐ Aspirin, or non-steroidal anti-inflammatory drugs (such as ibuprofen), and alcoholic beverages (within 24 hours after treatment)
☐ ☐ Under- or over-correction of wrinkles (resulting in poor aesthetic outcome)
☐ ☐ Patient's medical history
☐ ☐ Laser treatment, chemical peeling or other treatments based on active dermal response
☐ ☐ Dental procedures done near dermal fillers
Remark:

Attachment II: Hazards and contributing factors

This appendix provides a selection of categories of risks and subsequent examples, and is based on hazards described in the standard EN ISO 14971:2007, corrected 2012 Medical devices – Application of risk management to medical devices.

☐ Biological hazards
- Contamination with bacteria
- Contamination with viruses
- Contamination with endotoxins

☐ **Biocompatibility**
- Allergenicity/irritancy
- Cytotoxicity
- Acute systemic toxicity
- Subchronic toxicity
- Animal implantation
- Intracutaneous reactivity
- Genotoxicity
- Pyrogenicity
- Sensitization

☐ **Chemical hazards**
- Acids and alkalis
- Residues, e.g. cleaning
- Contaminating agents
- Manufacturing additives or adjuvants
- Degradation products
- Anaesthetic products

☐ **Use error**
- Routine violation
- Reasonably foreseeable use error
- Use by unskilled/untrained personnel
- Inadequate equipment
- Inadequate technique of injection/procedure
- Inadequate patients
- Injection into contraindicated areas
- Over-correction

☐ Hazardous phenomena resulting from incorrect energy and substance output
- Pressure
- Volume

☐ Hazardous phenomena linked to inadequate labelling
- Incomplete instructions for use
- Inadequate description of performance characteristics
- Inadequate specification of intended use
- Inadequate disclosure of limitations

☐ Hazardous phenomena linked to inadequate operating instructions
- Inadequate specification of accessories to be used with the medical device
- Incompatibility of consumables/accessories/other medical devices
- Inadequate specification of pre-use checks
- Over-complicated (operating) instructions

☐ Hazardous phenomena linked to insufficient warnings about
- Complications/side effects
- Hazards from re-using single use medical devices

☐ Incomplete requirements
- Inadequate specification of:
- design parameters
- performance requirements
- end of life
- plunger, needle, syringe

☐ Manufacturing processes
- Insufficient control of changes to manufacturing processes
- Insufficient control of materials/materials compatibility information
- Insufficient control of manufacturing processes
- Insufficient control of subcontractors

☐ Transport and storage
- Inadequate packaging
- Contamination or deterioration
- Inappropriate environmental conditions

☐ Environmental factors
- Physical, e.g. temperature, pressure, time
- Chemical, e.g. corrosions, degradation, contamination
- Mechanical, e.g. accidental mechanical damage

☐ Cleaning, disinfection and sterilization
- Lack of, or inadequate specification for, validated procedures for sterilization, and if applicable for cleaning and disinfection
- Inadequate conduct of cleaning, disinfection and sterilization

☐ Disposal and scrapping
- No or inadequate information provided

☐ **Formulation**
- (Bio)degradation
- Inadequate warning of hazards associated with incorrect formulations

☐ Potential for use errors triggered by design flaws, such as
- Missing instructions for use
- Ambiguous or unclear device state
- Ambiguous or unclear presentation of settings, measurements or other information

☐ **Failure modes**
- Unexpected loss of mechanical integrity
- Deterioration in function (e.g. change in resistance to flow) as a result of ageing
- Integrity and pressure resistance of the syringe
- Loss of sterility

Attachment III: Clinical evaluation of dermal fillers
It should be checked whether the headings given are addressed, not whether all items are addressed.

☐ **1. Indications**
- Facial areas
    - o Nasolabial folds
    - o Fine lines, rhytids
    - o Glabellar complex
    - o Brow elevation
    - o Lip augmentation and contouring
    - o Chin, cheek and temporal fossa augmentation
    - o Nose reshaping
    - o Tear trough
    - o Scars: traumatic, postacne, skin diseases
    - o Marionette lines
    - o Oral commissures
    - o Perioral, periocular lines and rhytids
    - o Prejowl sulcus
    - o Periocular melanoses
- Extrafacial areas
- Correction of HIV-associated facial lipoatrophy

☐ 2. Contraindications
See Attachment I – Contraindications
- Calcium hydroxylapatite is not appropriate for injecting in lips and for the nasojugal groove

☐ **3. Safety**
For (serious) adverse events see Attachment I – Complications/side effects

☐ **4. Performance**
- Wrinkle severity rating scale (WSRS) (a 5-point rating scale)
- Severity rating scale (SRS)
- Wrinkle assessment score (WAS) (a 6-point photo-numeric scale)
- Modified Fitzpatrick wrinkle scale (MFWS) (a 7-point rating scale on the basis of photographic images)
- Global aesthetic improvement scale (GAIS) (a 5-point rating scale for global aesthetic improvement appearance)
- Mid-face volume deficiency scale (MFVDS) (a 5-point rating scale; a 6-point rating scale)
- Medicis lip fullness scale (MLFS) (a 5-point rating scale)
- Satisfaction of the subject (questionnaire to judge the change in appearance of the subject after treatment)
- James scale (facial lipoatrophy scale) (a 4-point rating scale)

☐ 5. Duration of treatment effect
- Survival analysis is performed using Kaplan-Meier estimates to determine the duration of the effect of filler implantation based on the time point when the post-treatment score (e.g. MFVDS) returns to or is worse than the pre-treatment score
- Long-term treatment effect is described/analyzed

Annex 6: Results of the technical documentation assessment

Table 6.1. Assessment of the IFU

Id	Indications for use	Injection technique	DF-related risks	IFU in Dutch or English	Score
DF01	Y	P	Y	Y	M
DF02	Y	P	Y	Y	M
DF03	Y	P	Y	Y	M
DF04	Y	Y	Y	Y	G
DF05	Y	P	Y	Y	M
DF06	Y	Y	Y	Y	G
DF07	Y	P	Y	Y	M
DF08	Y	P	Y	Y	M
DF09	Y	P	Y	Y	M
DF10	Y	N	Y	Y	I
DF11	Y	Y	Y	Y	G
DF12	Y	Y	Y	Y	G
DF13	Y	Y	Y	Y	G

Grey-shaded sub-items represent major sub-items.
DF - dermal filler, Id - identifier, IFU - instructions for use.
Sub-item scores: N - no, P - partially, Y - yes.
Assessment scores: I - insufficient, M - moderate, G - good.

Table 6.2. Assessment of the risk analysis

Id	Risk management plan	Date/version risk analysis	DF-related risks	Risk categories	Risks estimated	Risk control/ mitigation	Acceptability residual risks	Conclusions	Score
DF01	Y	Y	Y	P	Y	Y	Y	Y	M
DF02	Y	Y	Y	Y	Y	Y	Y	Y	G
DF03	Y	Y	Y	P	Y	Y	Y	Y	M
DF04	Y	Y	Y	P	Y	Y	Y	Y	M
DF05	Y	Y	Y	P	Y	Y	Y	Y	M
DF06	Y	Y	Y	Y	Y	Y	Y	Y	G
DF07	Y	Y	Y	P	Y	Y	Y	Y	M
DF08	Y	Y	Y	Y	Y	Y	Y	Y	G
DF09	Y	Y	Y	Y	Y	Y	Y	Y	G
DF10	Y	Y	P	P	N	Y	Y	N	I
DF11	Y	Y	Y	P	Y	Y	Y	Y	M
DF12	Y	Y	Y	Y	Y	Y	Y	Y	G
DF13	Y	Y	Y	Y	Y	Y	Y	Y	G

Grey-shaded sub-items represent major sub-items.
DF - dermal filler, Id - identifier, IFU - instructions for use.
Sub-item scores: N - no, P - partially, Y - yes.
Assessment scores: I - insufficient, M - moderate, G - good.

Table 6.3. Assessment of the biocompatibility

Id	Literature review	Tests conducted	Appropriateness of tests	Standards applied	Test protocols	Summary of results	Conclusions	Score
DF01	N	Y	Y	Y	Y	Y	Y	M
DF02	N	Y	Y	Y	Y	Y	Y	M
DF03	N	Y	Y	Y	Y	Y	Y	M
DF04	N	Y	N	Y	Y	Y	Y	I
DF05	N	Y	Y	Y	Y	Y	Y	M
DF06	Y	Y	Y	Y	Y	Y	Y	G
DF07	P	Y	Y	Y	Y	Y	Y	M
DF08	P	Y	P	Y	Y	Y	Y	M
DF09	N	Y	Y	Y	Y	Y	Y	M
DF10	N	Y	Y	Y	Y	Y	Y	M
DF11	N	Y	P	Y	Y	Y	Y	M
DF12	N	Y	Y	Y	Y	N	N	I
DF13	Y	Y	P	Y	Y	Y	Y	M

DF - dermal filler, Id - identifier, IFU - instructions for use.
Sub-item scores: N - no, P - partially, Y - yes.
Assessment scores: I - insufficient, M - moderate, G - good.

Table 6.4. Assessment of the physical testing

Id	Tests conducted	Appropriateness of tests	Summary of results	Conclusions	Score
DF01	Y	P	Y	Y	M
DF02	Y	P	Y	Y	M
DF03	Y	P	Y	Y	M
DF04	Y	Y	Y	Y	G
DF05	Y	Y	Y	N	M
DF06	Y	Y	Y	Y	G
DF07	Y	P	Y	Y	M
DF08	Y	Y	Y	Y	G
DF09	Y	P	Y	Y	M
DF10	Y	P	Y	Y	M
DF11	Y	Y	N	N	I
DF12	Y	Y	Y	Y	G
DF13	Y	P	Y	Y	M

DF - dermal filler, Id - identifier, IFU - instructions for use.
Sub-item scores: N - no, P - partially, Y - yes.
Assessment scores: I - insufficient, M - moderate, G - good.

Table 6.5. Assessment of the clinical evaluation

Id	Rationale for equivalence	Name of medical device	Identifi-cation of Manu-facturer	Description of medical device	Indications	Safety or performance claims	Objective of evaluation	Choice of clinical data types substantiated
DF01	NA	Y	Y	Y	P	P	Y	Y
DF02	P	Y	Y	Y	Y	P	Y	Y
DF03	NA	Y	Y	Y	Y	P	Y	Y
DF04	Y	Y	Y	Y	Y	N	Y	Y
DF05	Y	Y	Y	N	N	N	Y	Y
DF06	Y	Y	Y	Y	Y	N	Y	Y
DF07	NA	Y	Y	Y	Y	N	Y	Y
DF08	Y	Y	Y	Y	Y	Y	Y	Y
DF09	Y	Y	Y	Y	Y	N	Y	Y
DF10	P	Y	Y	Y	Y	Y	Y	N
DF11	Y	Y	Y	Y	Y	N	Y	Y
DF12	NA	Y	Y	Y	Y	Y	Y	Y
DF13	NA	Y	Y	Y	Y	Y	Y	Y

Id	Literature search strategy	Clinical follow-up	Perfor-mance analysis	Safety analysis	Relevant topics	Summary of clinical data and appraisal	Conclus ions	Score
DF01	Y	Y	Y	Y	Y	Y	Y	M
DF02	Y	Y	Y	Y	Y	Y	Y	M
DF03	Y	Y	Y	Y	Y	Y	Y	M
DF04	Y	Y	Y	Y	Y	P	Y	I
DF05	P	Y	P	P	Y	P	Y	I
DF06	Y	Y	Y	Y	Y	P	Y	I
DF07	Y	Y	Y	Y	Y	P	Y	I
DF08	P	Y	Y	Y	Y	Y	Y	M
DF09	Y	Y	Y	Y	P	P	Y	I
DF10	P	Y	P	P	P	P	Y	I
DF11	Y	Y	Y	Y	Y	Y	Y	M
DF12	Y	Y	Y	Y	Y	Y	Y	G
DF13	Y	Y	Y	Y	Y	P	Y	M

Grey-shaded sub-items represent major sub-items.

DF - dermal filler, Id - identifier, IFU - instructions for use.

Sub-item scores: N - no, NA - not applicable, P - partially, Y - yes.

Assessment scores: I - insufficient, M - moderate, G - good.

Table 6.6. Assessment of the summary and analysis of PMS data

Id	PMS sources identified	Analysis PMS data	Summary PMS data and conclusions	Actions taken	Score
DF01	Y	Y	C	Y	G
DF02	Y	Y	C	Y	G
DF03	Y	Y	C	Y	G
DF04	Y	N	N	N	I
DF05	Y	Y	C	Y	G
DF06	Y	Y	L	Y	M
DF07	Y	Y	C	Y	G
DF08	Y	Y	C	Y	G
DF09	Y	Y	C	Y	G
DF10	Y	Y	C	Y	G
DF11	Y	Y	C	Y	G
DF12	Y	Y	L	Y	M
DF13	Y	Y	C	Y	G

DF - dermal filler, Id - identifier, IFU - instructions for use.
Sub-item scores: C - clear, L - limited, N - no, P - partially, Y - yes.
Assessment scores: I - insufficient, M - moderate, G - good.

Annex 7: Products received in market surveillance

Filler	Same product as*	Material	Exp. date	Lidocaine	Analyzed by
A097301		hyaluronic acid	Jan-17	no	NMR/LC-MS
A097302	A097301	hyaluronic acid	Sep-17	no	NMR/LC-MS
A097303		hyaluronic acid	Mar-17	no	NMR/LC-MS
A097304	A097303	hyaluronic acid	Mar-17	no	NMR/LC-MS
A097305	A097303	hyaluronic acid	Aug-17	no	NMR/LC-MS
A097306		Ca hydroxylapatite	may-17	no	SEM-EDX
A097307	A097306	Ca hydroxylapatite	Aug-17	no	SEM-EDX
A097308	A097306	Ca hydroxylapatite	Jul-17	no	-
A097309		hyaluronic acid	Oct-16	no	NMR/LC-MS
A097310	A097309	hyaluronic acid	May-17	no	NMR/LC-MS
A097311		hyaluronic acid	Jul-16	no	NMR/LC-MS
A097312	A097311	hyaluronic acid	Jun-17	no	NMR/LC-MS
A097313	A097311	hyaluronic acid	Jun-17	no	NMR/LC-MS
A097314		hyaluronic acid	Jan-17	no	NMR/LC-MS
A097315	A097314	hyaluronic acid	Jan-17	no	NMR/LC-MS
A097316	A097314	hyaluronic acid	Jan-17	no	NMR/LC-MS
A097317		hyaluronic acid	Dec-17	no	NMR/LC-MS
A097318	A097317	hyaluronic acid	Dec-17	no	NMR/LC-MS
A097319	A097317	hyaluronic acid	Nov-17	no	NMR/LC-MS
A097320		hyaluronic acid	Aug-17	no	NMR/LC-MS
A097321	A097320	hyaluronic acid	Sep-17	no	NMR/LC-MS
A097322	A097320	hyaluronic acid	Oct-17	no	NMR/LC-MS
A097323		hyaluronic acid	Jun-16	no	NMR/LC-MS
A097324	A097323	hyaluronic acid	Jul-16	no	NMR/LC-MS
A097325	A097323	hyaluronic acid	mrt-17	no	NMR/LC-MS
A097326		hyaluronic acid	Jun-17	no	NMR/LC-MS
A097327	A097326	hyaluronic acid	Oct-17	no	NMR/LC-MS
A097328	A097326	hyaluronic acid	Jan-18	no	NMR/LC-MS
A097329		hyaluronic acid	Jul-17	no	NMR/LC-MS
A097330	A097329	hyaluronic acid	Nov-17	no	NMR/LC-MS
A097331	A097329	hyaluronic acid	Nov-17	no	NMR/LC-MS
A097401		hyaluronic acid	Dec-17	yes	NMR/LC-MS
A097402	A097401	hyaluronic acid	Jan-18	yes	NMR/LC-MS
A097403	A097401	hyaluronic acid	Jan-18	yes	NMR/LC-MS
A097404		hyaluronic acid	Dec-17	yes	NMR/LC-MS
A097405	A097404	hyaluronic acid	Dec-17	yes	NMR/LC-MS
A097406	A097404	hyaluronic acid	Dec-17	yes	NMR/LC-MS
A097407		hyaluronic acid	May-17	yes	NMR/LC-MS

Filler	Same product as*	Material	Exp. date	Lidocaine	Analyzed by
A097408	A097407	hyaluronic acid	Aug-17	yes	NMR/LC-MS
A097409	A097407	hyaluronic acid	Aug-17	yes	NMR/LC-MS
A097410		hyaluronic acid	Aug-17	yes	NMR/LC-MS
A097411	A097410	hyaluronic acid	Sep-17	yes	NMR/LC-MS
A097412	A097410	hyaluronic acid	Aug-17	yes	NMR/LC-MS
A097413		hyaluronic acid	May-18	yes	NMR/LC-MS
A097414	A097413	hyaluronic acid	Nov-18	yes	NMR/LC-MS
A097415	A097413	hyaluronic acid	Nov-18	yes	NMR/LC-MS
A097416		hyaluronic acid	Nov-17	no	NMR/LC-MS
A097417	A097416	hyaluronic acid	Jun-17	no	NMR/LC-MS
A097418	A097416	hyaluronic acid	Nov-17	no	NMR/LC-MS
A097419		poly-ε-caprolacton	Jun-17	no	NMR/LC-MS/SEM-EDX
A097420	A097419	poly-ε-caprolacton	Aug-17	no	NMR/LC-MS/SEM-EDX
A097421	A097419	poly-ε-caprolacton	Sep-17	no	NMR/LC-MS
A097422		poly-ε-caprolacton	Oct-17	no	NMR/LC-MS/SEM-EDX
A097423	A097422	poly-ε-caprolacton	Oct-17	no	NMR/LC-MS/SEM-EDX
A097424	A097422	poly-ε-caprolacton	Oct-17	no	NMR/LC-MS
A097425		poly-L-lactic acid	Sep-18	no	SEM-EDX
A097426	A097425	poly-L-lactic acid	Sep-17	no	SEM-EDX
A098301		hyaluronic acid	Apr-16	no	NMR/LC-MS
A098302	A098301	hyaluronic acid	Nov-17	no	NMR/LC-MS
A098303	A098301	hyaluronic acid	Nov-16	no	NMR/LC-MS
A098304		hyaluronic acid	Nov-17	no	NMR/LC-MS
A098305	A098304	hyaluronic acid	Nov-17	no	NMR/LC-MS
A098306	A098304	hyaluronic acid	Aug-17	no	NMR/LC-MS
A098307		hyaluronic acid	Aug-18	no	NMR/LC-MS
A098308	A098307	hyaluronic acid	Oct-18	no	NMR/LC-MS
A098309	A098307	hyaluronic acid	Nov-18	no	NMR/LC-MS
A098310		hyaluronic acid	Sep-18	no	NMR/LC-MS
A098311	A098310	hyaluronic acid	Sep-18	no	NMR/LC-MS
A098312	A098310	hyaluronic acid	Dec-18	no	NMR/LC-MS
A098313		hyaluronic acid	mrt-18	no	NMR/LC-MS
A098314	A098313	hyaluronic acid	mrt-18	no	NMR/LC-MS
A098315	A098313	hyaluronic acid	Jun-18	no	NMR/LC-MS
A098316		hyaluronic acid	mrt-18	no	NMR/LC-MS
A098317	A098316	hyaluronic acid	mrt-18	no	NMR/LC-MS
A098318	A098316	hyaluronic acid	mrt-18	no	NMR/LC-MS

All requested products were received

* Received products obtained an unique A-number at arrival at the RIVM. Since products were provided in duplicate or triplicate, certain A-numbers represent the same product, which is reflected in this column

Annex 8: Physicochemical methods

Digestion of hyaluronic acid based fillers
An amount of 90 mg of dermal filler is dissolved in 900 µL of 10 mM ammonium acetate by putting it 30 minutes on a roller-bank at ambient temperature. 200 µL of this solution is placed into a new vial and digested by adding 10 µl of enzyme solution (10 UN Chondroitinase AC in 833 µl of 10 mM ammonium acetate and 1 mg/mL bovine serum albumine), and placing the vials for 24 hours at 37 °C, with a shaking frequency of 150 rpm. As a positive control, 30 mg of 10 mg hyaluronic acid in 0.5 mL of 10 mM ammonium acetate buffer is digested under identical conditions.

LC-MS analysis
For the chromatography, an Agilent 1100 system was used equipped with a Superdex Peptide 10/300 column (GE Healthcare), a flow of 0.5 mL/min of 12mM ammonium acetate buffer (pH 9) and a runtime of 60 minutes. The column was hyphened to a LCQ-Deca-XP (Thermo) mass spectrometer using a full scan mode from 300-1500 amu in the negative mode. The diverting valve to the waste is used the first 10 minutes and the last 17 minutes of the run. Fragments are identified according to the assignments in (refs Kenne and Kuhn). Peak areas were determined using Xcalibur 2.0 SR2 software (Thermo). For the calculation of the modification grade, the peak surfaces of all blank corrected BDDE containing fragments were summed and divided by the sum of all hyaluronic acid fragments. The modification is expressed as a percentage. The cross-linking grade is calculated as the percentage of the peak surfaces of all blank corrected saccharide-BDDE-saccharide fragments divided by the total area of hyaluronic acid fragments.

NMR spectroscopic analysis
200 µl of product digests were diluted with 200 µl D_2O before NMR spectroscopy. ^1H spectra were acquired at 14.1 T on a Bruker DMX 600 MHz spectrometer (Bruker, Wormer, the Netherlands) equipped with a TCI-Z-GRAD cryoprobe operating at 298 K. All samples were automatically tuned, matched and shimmed. Spectra were calibrated to the solvent peak of HDO (4.7 ppm). Spectra were processed and analysed using Topspin 3.0 software (Bruker, Wormer, the Netherlands). Lidocaine was identified by the presence of signals at 1.2724 and 2.1653 ppm.

SEM-EDX analysis
Samples were spotted, smeared and left to dry at ambient temperature on carbon pads. A Zeiss EVO LS 10 microscope was used, operated by Zeiss SmartSEM 5.7.SP2 software with a typical accelerating voltage of 20 kV. The beam size was 400 pA, the working distance: 8.5 mm, a low pressure vacuum of around 30 Pa (N_2) was used and as detectors a variable pressure secondary electron detector (VPSED) and a high definition backscattering detector (HDBSD). For the elemental analysis, an Oxford Instruments X-MAX 50 was used, operated by AZTec 3.0.SP2 software with a process time of 6 at the lowest and a pixel dwell time of 50 µs. Total counts in a mapping were at least 3.14 million and total

counts in a point analysis were at least 500.000. Particle size was determined using ImageJ 1.51g (NIH).

Annex 9: Biocompatibility methods

Sample preparation
Biocompatibility was assessed by evaluating the cytotoxic potential for in total 26 fillers of 14 brands. Two batches of each filler type were tested, except for one manufacturer for which one batch of one type of filler was tested and for one manufacturer for which three batches of one type of filler were tested. According to ISO 10993-12 [27], hydrophilic extracts were prepared of the dermal filler materials by incubating 0.2 g/mL using tissue culture medium in the presence of fetal bovine serum (incubation for 72 ± 2 hours at 37 ± 1 °C). The extracts were incubated for 72 hours, the longest period of extraction indicated in ISO 10993-5, in order to allow an optimal possibility for extraction. A temperature of 37°C was chosen because a higher temperature of 50°C (as indicated in ISO 10993-5) might affect the filler material (e.g. degradation). Also 37°C is the body temperature of persons in which the fillers are used. After 72 hours of extraction, the extracts were filtered with 0.22 µm Spin-X centrifuge tube filters (Corning, Cat No 8160) by centrifugation at 16,000g for 20 minutes and evaluated for biocompatibility in a cytotoxicity assay. For one of the preparations (A098307) an alternative experiment was performed. This filler product is an aqueous non cross-linked preparation, which gave a viscous extract that remained filtering with 0.22 µm Spin-X centrifuge tube filters. Therefore, the extracts were prefiltered with Vivaspin 500, 100,000 MWCO PES (Sartorius, Cat No VS0141) and centrifuged at 15,000g for 20 minutes. Since fine-tuning the experimental set-up finished the filler material, additional product material (of the same batch) was requested at the manufacturer. Therefore, this filler product received new A-numbers, being A115701, A115702 and A115703. Also from the second type of filler from this manufacturer (initial A-number A098310) additional product material was obtained, corresponding to the new A-numbers A115704, A115705 and A115706. For this experiment additional controls were included being increased doses of DMSO (up to 16%) and a highly toxic extract of Sn stabilized PVC [31]. A115704, A098313, and A097312 were included in order to compare with cross-linked fillers. A097420 was included for comparison with a non-hyaluronic acid filler.

Cell culture and cytotoxicity assay
The cytotoxicity assay was performed according to international standard ISO 10993-5 [24], describing cytotoxicity assays with hydrophilic extracts (e.g. tissue culture medium). Cell cultures of a macrophage cell line (RAW264.7) and a fibroblast cell line (L929) were incubated with the filtered extract for 24 hours. After the incubation period the extract was removed, the cells were rinsed once and the cell viability and membrane integrity was determined by incubating the cells with a mixture of Alamar Blue/CFDA-AM (5-carboxyfluorescein diacetate, acetoxymethyl ester). Alamar Blue conversion depends on the metabolic activity of the cells and as such indicates cell viability, while CDFA-MA uptake and conversion to a fluorescent dye reflects the presence as well as the amount of esterases within the cell and as such indicates membrane integrity.

RAW264.7 and L929 murine cells were cultured in a 75 cm^2 tissue culture flask to propagate the cells. The culture medium for both cell cultures was Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12, without phenol red, Gibco, Cat No 21041) supplemented with 10% fetal bovine serum (FBS, Greiner Bio-One, Cat No 758093), 1% sodium pyruvate (Gibco, Cat No 11360) and 100 U/mL penicillin, 100 µg/mL streptomycin (Gibco, Cat No 15070063).

Cells in the exponential growth phase were isolated, counted and seeded in black flat-bottom 96-well cell culture plates at 2x 10^4 cells per well for RAW264.7 cells and 1x 10^4 cells per well for L929 cells. After 20 hours the cells formed a semi-confluent monolayer and 100 µl of filtered extract was added and incubated for 24 hours. Then 65 µL extract was pipetted off, cells were carefully rinsed with 180 µL DPBS (Gibco Cat No 14190) and cell viability was determined by using a combined Alamar Blue/CFDA-AM (AB/CFDA) assay (Alamar Blue, Roche Cat No DAL1025, CFDA-AM, Molecular Probes, Cat No C1354). The AB/CFDA protocol was adapted from Heusinkveld et al. [28] and Lammel et al. [29], to simultaneously determine both mitochondrial activity and membrane integrity. Mitochondrial activity of the cells was recorded as an indication for cell viability with the AB assay, which is based on the ability of the cells to reduce resazurin to the red bright fluorescent resorufin. In the same experiment, membrane integrity was assessed indirectly using a CFDA-AM assay, which is based on nonspecific cytoplasmic-esterase activity transforming the CFDA-AM into a fluorescent product. A reduced presence of fluorescence in the cells indicates dead or damaged cells by membrane leakage of the unhydrolysed CFDA-AM substrate and the fluorescent product. Briefly, cells were incubated with 4µM CFDA-AM and a ten-fold dilution of Alamar Blue in DMEM/F12, after 1 hour hydrolysed CF (carboxy fluorescein) was measured spectrophotometrically at 485/535 nm, whereas resorufin was measured spectrophotometrically at 530/590 nm after 3 hours, both measurements were performed in a SpectraMax M2 spectrophotometer (Molecular Devices). The amount of resorufin formed and the measured fluorescence directly correlates to the number of viable cells.

The cytotoxicity assay was performed in four-fold (i.e. four cell culture wells were incubated with 100 µL extract), as positive control DMSO in tissue culture medium was used: 2% for RAW264.7 and 5% for L929 cells. Cell survival of treated cells was expressed relative to the viability of non-treated (medium only) control cells. The alternative experiment with A115701 was performed with L929 cells only. As the 5% DMSO control showed moderate toxicity for L929 cells (see Figure 5.2 and Table 10.1), in this experimental set-up also higher concentrations of DMSO (up to 16%) and a highly toxic extract of Sn stabilized PVC [31] were used as positive controls.

Annex 10: Results of the biocompatibility screening

Table 10.1. Cytotoxic activity in response to dermal filler extracts

Exposure conditions[1]	**Cell viability in %**		**Cell membrane integrity in %**	
	RAW264.7	**L929**	**RAW264.7**	**L929**
Medium[2]	100 ± 2 (36)	100 ± 3 (36)	100 ± 3 (36)	100 ± 7 (36)
Medium_20%PBS	95 ± 2 (20)	97 ± 3 (24)	102 ± 5 (20)	101 ± 5 (24)
DMSO[3]	**60 ± 16 (25)**	**50 ± 4 (30)**	89 ± 4 (25)	89 ± 7 (30)
A097301	87 ± 1 (4)	94 ± 3 (4)	107 ± 1 (4)	101 ± 3 (4)
A097303	91 ± 2	94 ± 2	103 ± 2	100 ± 3
A097309	84 ± 2	90 ± 3	99 ± 3	100 ± 7
A097311	82 ± 3	92 ± 3	99 ± 4	96 ± 5
A097314	90 ± 2	86 ± 3	100 ± 2	97 ± 2
A097318	90 ± 2	88 ± 6	102 ± 1	99 ± 5
A097320	95 ± 2	91 ± 3	101 ± 1	100 ± 2
A097324	90 ± 2	85 ± 5	101 ± 2	98 ± 4
A097326	97 ± 3	96 ± 3	100 ± 3	97 ± 3
A097329	91 ± 2	99 ± 3	98 ± 2	101 ± 6
A097401	97 ± 3	92 ± 3	101 ± 1	99 ± 2
A097404	99 ± 2	93 ± 5	100 ± 2	98 ± 2
A097407	97 ± 3	89 ± 5	102 ± 2	99 ± 6
A097410	99 ± 3	95 ± 5	103 ± 2	99 ± 8
A097413	102 ± 3	98 ± 2	99 ± 3	100 ± 4
A097416	94 ± 3	95 ± 2	103 ± 3	99 ± 3
A098301	86 ± 3	94 ± 3	103 ± 3	96 ± 8
A098304	92 ± 2	93 ± 4	101 ± 3	95 ± 4
A098310	88 ± 3	88 ± 2	103 ± 2	101 ± 9
A098313	86 ± 2	89 ± 2	104 ± 3	101 ± 6
A098316	86 ± 4	88 ± 3	105 ± 3	100 ± 6
A097306	93 ± 3	88 ± 4	100 ± 3	93 ± 4
A097419	78 ± 3	96 ± 2	95 ± 6	96 ± 6
A097422	81 ± 3	99 ± 2	95 ± 4	93 ± 7
A097425	93 ± 2	98 ± 3	96 ± 6	95 ± 5

[1] Exposure conditions consist of positive controls (medium and DMSO) or a filler extract.
[2] Cell viability and membrane integrity after incubation in medium alone (non-treated control) was set at 100%.
[3] DMSO added as positive cytotoxic control: 2% for RAW264.7 cells and 5% for L929 cells.
Highlighted: cell viability/membrane integrity below 70%, reflecting cytotoxic activity.
The last four products represent findings on non-hyaluronic acid-based fillers.
All data are the mean of n=8 measurements, unless otherwise indicated within brackets.

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