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BY HAND DELIVERY

Dockets Management Branch (HFA-305)
Food and Drug Administration
Department of Health and Human Services
5630 Fishers Lane, Room 1061
Rockville, Maryland 20852

CITIZEN PETITION SUPPLEMENT No. 3
(03P-0064)

The undersigned, on behalf of sanofi-aventis US LLC, a subsidiary of sanofi-aventis, and successor in interest to Aventis Pharmaceuticals, S.A. ("sanofi-aventis") submits this Citizen Petition Supplement No. 3 to its Citizen Petition filed February 19, 2003 (03P-0064/CPI) (hereafter the "Citizen Petition"). This Supplement No. 3 is submitted under Sections 505(b) and 505(j) of the Federal Food, Drug, and Cosmetic Act ("FDCA") (21 U.S.C. §§ 355(b) and (j)) and 21 C.F.R. § 10.30. This Supplement No. 3 is supported by signed Declarations of Jeffrey I. Weitz, M.D., and Jeremy E. Turnbull, Ph.D., which are attached hereto in Appendix A.

I. Summary

The Citizen Petition requests that until such time as enoxaparin has been fully characterized, FDA refrain from approving any ANDA citing Lovenox®as the reference listed drug unless the manufacturing process used to create the generic product is determined to be equivalent to sanofi-aventis' manufacturing process for enoxaparin, or the application is supported by proof of equivalent safety and effectiveness demonstrated through clinical trials.1 Sanofi-aventis now provides this Supplement No. 3 to describe the minimum requirements for adequate and full characterization of enoxaparin. In the process, it provides important new information about the distinctive (and process dependent) biological properties of enoxaparin, revealed through sanofi-aventis' continuing investigation into the structure of enoxaparin. Finally, the Supplement addresses issues regarding demonstrations of sameness.

Enoxaparin derives its biological and clinical activities from the structures of its component polysaccharides (which structures are dependent upon the process sanofi-aventis uses to manufacture enoxaparin). Yet little is known about how the particular structural features of these polysaccharides cause enoxaparin's distinctive spectrum of biological and clinical effects,
or how the sanofi-aventis manufacturing process influences those structural features. Through its ongoing research, however, sanofi-aventis has discovered that small differences in the structure of particular polysaccharide chains can lead to important differences in biological activity, which may have clinical significance.

As a result, to fully characterize enoxaparin, one must identify and structurally characterize each of enoxaparin’s unique polysaccharide chains and determine its relative abundance. Any less rigorous approach could fail to ensure that important structural features that may make clinically significant contributions are also present in a generic product. For example, attempts to characterize enoxaparin through reference to certain limited structural features or biological activities, as discussed below, may provide some information about a limited set of enoxaparin’s characteristics, but would fail to address other perhaps equally important biologically and clinically relevant characteristics.2

II. Full Characterization of Enoxaparin Requires Sequencing Each Unique Polysaccharide Chain

A. Enoxaparin Is a Highly Complex Mixture, With High Variability From One Polysaccharide Chain to Another

Enoxaparin is a mixture of many different types of polysaccharide molecules, each of which is a polymer composed of a linear chain of sugars.4 Enoxaparin polysaccharides range in length from 2 sugars to 26 (or even more) sugars (i.e., 2mers to 26mers, or larger, such as 32mers). There are many different kinds of component sugars in enoxaparin, some of which are natural and some of which are specifically created during the enoxaparin manufacturing process.5 Each unique polysaccharide chain is composed of a specific subset of the available component sugars, which are arranged in a particular order from one end of the polysaccharide chain to the opposite end. In other words, each unique polysaccharide in the enoxaparin mixture has a different sequence of component sugars.

The overall enoxaparin mixture is particularly complex because as chain length increases, the number of potentially unique sequences increases exponentially. For instance, a

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4 The term “sugar,” as used herein, refers to a simple sugar or monosaccharide residue (1mer).
5 In general, enoxaparin polysaccharides are conveniently viewed as polymers composed of a variety of different disaccharide (2mer) building blocks linked together in a linear repeating fashion. That is because the polymers of heparin, the natural product from which enoxaparin is derived, are synthesized from disaccharide (2mer) building blocks. Some have commented that there may be up to 48 different disaccharide building blocks in enoxaparin. See Pacific Growth Equities 2006 Life Sciences Growth Conference - Final FD (Fair Disclosure) Wire June 14, 2006 (LEXIS, Transcripts Library, 061406ac.700). However, a number of those 48 disaccharides have not been reported. More conservatively, one study estimates that there are at least twelve different disaccharide building blocks in heparin, the natural product from which enoxaparin is derived. See Behr JR, et al. Quantification of isomers from a mixture of twelve heparin and heparan sulfate disaccharides using tandem mass spectrometry. Rapid Commun. Mass Spectrom. 2005; 19:2553-2562 at 2555. In addition, enoxaparin contains chemically-modified, process-dependent monosaccharides such as galacturonic acid, mannosamine, and other chemical modifications such as a 1,6 anhydro ring structure at the reducing end of certain polysaccharides. See Citizen Petition, at 12-13. While the building blocks of enoxaparin polysaccharides are normally viewed as disaccharides, enoxaparin’s manufacturing process also leads to the production of odd-numbered polysaccharide chains. See Citizen Petition, at 12.
simple mathematical model of the total number of octasaccharide (8mer) sequences in an LMWH such as enoxaparin is the number of possible different building blocks statistically available to build those octasaccharides raised to the power of the number of disaccharide building blocks needed to make an octasaccharide, which is four. Assuming that, on average, only three different building blocks were statistically available (which sanofi-aventis believes to be a conservative estimate), the number of different octasaccharides would be $3^4$, or 81. Similarly, the total number of dodecasaccharide (12mer) sequences would be $3^6$, or 729. The total number of possible 20mer polysaccharide sequences would be $3^{10}$, or 59,049, and the total number of possible 26mer polysaccharide sequences would be $3^{13}$, or 1.6 million.

**B. The Biological Activities of Enoxaparin Are Determined by The Biological Activity of Each Unique Polysaccharide and the Relative Abundances of Those Polysaccharides**

Enoxaparin's complex mixture of different polysaccharide molecules produces complex biological and clinical activities. For example, enoxaparin has antithrombotic activity due to its actions on several proteins including antithrombin III (“ATIII”), Factors IIa, Xa, and VIIa, as well as on tissue factor pathway inhibitor (TFPI), among others. Enoxaparin also acts as an anti-inflammatory agent, in part from its effects on components of the contact and complement systems, discussed below, and on selectins.

The sequences of the different polysaccharides in enoxaparin, and the relative amounts of those different polysaccharides in the enoxaparin mixture, directly determine the biological and clinical activities of enoxaparin. However, the specific polysaccharide sequences that cause these biological effects are poorly understood or, in many cases, unknown. Accordingly, the only methods that can ensure that a generic product would have the same biological and clinical effects as enoxaparin are to characterize and compare all of the different polysaccharide sequences and their relative amounts, demonstrate the use of an equivalent manufacturing process, or perform clinical trials.

**1. Even Enoxaparin’s Most Studied Activities Are Influenced by Small Differences in Unique Polysaccharide Chains**

One of the most studied enoxaparin-protein interaction is the binding of certain enoxaparin polysaccharides to ATIII. In its Citizen Petition, sanofi-aventis noted that LMWHs, like unfractionated heparin, inhibit the coagulation cascade in part by binding to ATIII via a particular pentasaccharide sequence distributed along some of the polysaccharide chains, which we term herein “the classical ATIII-binding sequence.” Recent data suggest, however, that the

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ATIII binding affinity of that classical ATIII-binding sequence is influenced by the nature of the particular saccharide units flanking it in the different polysaccharide chains.\textsuperscript{6}

Sanofi-aventis has recently isolated and identified twenty-one process-dependent, pure ATIII-binding polysaccharides from enoxaparin, which range in length from 6mers to 10mers. Dr. C. Boudier of Université Louis Pasteur de Strasbourg in Illkirch, France determined the \textit{in vitro} ATIII binding affinities of those polysaccharides. Dr. Boudier’s results are presented in summary form below in Tables 1 and 2 and in detail in Appendix B.

Ten of the 21 newly-identified polysaccharides contain the classical ATIII binding sequence as part of their overall sequence. Nevertheless, as shown in Table 1, the ATIII binding affinities of those 10 polysaccharides vary over a wide range, with $K_d$ values of 1.49 to 567.60 nM. Lower $K_d$ values in Table 1 correspond to tighter ATIII binding while higher $K_d$ values correspond to weaker ATIII binding.
Table 1

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>$K_d$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>311.90</td>
</tr>
<tr>
<td>8$^b$</td>
<td>208.50</td>
</tr>
<tr>
<td>9</td>
<td>567.60</td>
</tr>
<tr>
<td>11</td>
<td>21.10</td>
</tr>
<tr>
<td>12</td>
<td>52.80</td>
</tr>
<tr>
<td>13</td>
<td>196.20</td>
</tr>
<tr>
<td>15</td>
<td>42.40</td>
</tr>
<tr>
<td>19</td>
<td>26.30</td>
</tr>
<tr>
<td>20$^c$</td>
<td>466.60</td>
</tr>
<tr>
<td>21</td>
<td>1.49</td>
</tr>
<tr>
<td>Synthetic Pentasaccharide Control$^d$</td>
<td>20.80</td>
</tr>
</tbody>
</table>

$^a$ Each of the compounds is a pure polysaccharide containing the classical ATIII-binding sequence. The compounds range in size from a 7mer to a 10mer.

$^b$ Polysaccharide 8 contains a natural variant of the classical ATIII-binding sequence having an N-sulfated glucosamine moiety rather than an N-acetylated glucosamine moiety.

$^c$ Polysaccharide 20 contains a 1,6 anhydro ring structure at the reducing end of a classical ATIII-binding sequence.

$^d$ A synthetic pentasaccharide with the sequence of Arixtra® (aka. fondaparinux; GlaxoSmithKline) is used as a control. Like compound 8, that pentasaccharide has an N-sulfated glucosamine moiety.

The observed, wide variation in ATIII-binding affinity results from the different sets of monosaccharides that flank the classical ATIII-binding sequence in each polysaccharide. For example, compound 21 is structurally similar to an octasaccharide that sanofi-aventis previously described in Citizen Petition Supplement No. 1 ($\Delta$Ia-IIa-IIg-I$. The two octasaccharides differ only in the configuration of the uronic acid of the IIa-disaccharide unit located just outside the bounds of the classical ATIII-binding sequence.$^e$

$^d$ See Citizen Petition Supplement No. 1, at 6. $K_d = 334.0$ nM (SE = 82%; 1st series); $K_d = 120$ nM (SE = 18%; 2nd series).

$^e$ The uronic acid of the IIa disaccharide unit of compound 21 is glucuronic acid, rather than iduronic acid as in $\Delta$Ia-IIa-IIg-I$. 
That tiny structural change in compound 21 results in an at least 100-fold difference in ATIII-binding affinity between compound 21 and Δls-IIa-IIg-Is. That result unexpectedly shows that even a small structural change in a monosaccharide residue adjacent to the classical ATIII-binding sequence can have large effects on ATIII-binding affinity. Sanofi-aventis has observed that the relative concentration of compound 21 and Δls-IIa-IIg-Is (both present in enoxaparin) is sensitive to the manufacturing process, and thus constitutes a process-dependent fingerprint of enoxaparin.

This finding highlights the fact that full characterization requires sequencing of all of enoxaparin's polysaccharide chains. Any attempt at characterization that stopped short of this level of detail would likely fail to account for the context-dependent variations at the individual polysaccharide level discussed above. A generic product based on such an incomplete characterization might therefore exhibit an antithrombotic profile different from that of enoxaparin.

The data presented above also highlight the shortcomings of reported techniques for demonstrating sameness to enoxaparin. For example, researchers collaborating with one ANDA applicant have published a method for detecting a purported surrogate for the classical ATIII-binding sequence. That surrogate is a tetrasaccharide comprising only three of the five monosaccharides of the classical ATIII-binding sequence, together with one flanking monosaccharide, as depicted in Figure 1.

It is evident from Figure 1 that the purported surrogate cannot adequately predict ATIII-binding activity for at least two reasons. First, the purported surrogate detects only three of the five sugars of the classical ATIII-binding sequence. (See the two boxes marked with question marks in Figure 1.) Accordingly, the purported surrogate cannot distinguish a classical ATIII-binding sequence from other sequences having the detected three-sugar unit but lacking the necessary fourth and fifth sugars. Second, the purported surrogate contains one sugar at the non-reducing end of the chain that is not part of the classical ATIII-binding sequence (see the grey box immediately to the left of the five white boxes) while failing to take into account the saccharide at the other end of the classical ATIII-binding sequence. Therefore, the purported surrogate would fail to recognize classical ATIII-binding sequences that are located adjacent to different types of sugars on either side. Thus it does not accurately reflect either the amount of classical ATIII-binding sequence in a given LMWH or the variability in ATIII-affinity caused by the particular monosaccharides flanking the classical ATIII-binding sequence.
2. The Classical ATIII-Binding Sequence Is Not Necessary for High-Affinity ATIII Binding

Additional data from Dr. Boudier demonstrate that enoxaparin polysaccharides can have high ATIII-binding affinity even though they lack the classical ATIII-binding sequence. This is a surprising new discovery that further underscores the need to characterize all polysaccharides in enoxaparin.

Dr. Boudier's results surprisingly demonstrate that the classical ATIII-binding sequence is not the only structural motif in enoxaparin that binds to ATIII with high affinity. Eleven of the polysaccharides that Dr. Boudier studied, reported in Table 2 below, do not contain the classical ATIII-binding sequence but nonetheless bind to ATIII with the same range of affinities as the polysaccharides reported in Table 1, which do contain the classical ATIII-binding sequence. In other words, enoxaparin possesses polysaccharide sequences that do not contain the classical ATIII-binding sequence, but nevertheless bind to ATIII with high affinity.

Specifically, the ATIII-binding affinities of those 11 polysaccharides vary widely, with $K_d$ values ranging from 1.15 to ~1300 nM, as shown in Table 2:

<table>
<thead>
<tr>
<th>Compound No. a</th>
<th>$K_d$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>303.50</td>
</tr>
<tr>
<td>2</td>
<td>114.30</td>
</tr>
<tr>
<td>4</td>
<td>203.20</td>
</tr>
<tr>
<td>5</td>
<td>1,338.20</td>
</tr>
<tr>
<td>6</td>
<td>1.15</td>
</tr>
<tr>
<td>7</td>
<td>306.50</td>
</tr>
<tr>
<td>10</td>
<td>142.90</td>
</tr>
<tr>
<td>14</td>
<td>63.60</td>
</tr>
<tr>
<td>16</td>
<td>80.40</td>
</tr>
<tr>
<td>17</td>
<td>92.10</td>
</tr>
<tr>
<td>18</td>
<td>184.40</td>
</tr>
<tr>
<td>Synthetic Pentasaccharide Control b</td>
<td>20.80</td>
</tr>
</tbody>
</table>

a Each of the compounds is a pure polysaccharide that does not contain the classical ATIII-binding sequence. The compounds in Table 2 range in size from a 6mer to a 10mer.

b A synthetic pentasaccharide with the sequence of Arixtra® is used as a control. That pentasaccharide has an $N$-sulfated glucosamine moiety.
Indeed, one of those polysaccharides, octasaccharide 6, binds to ATIII with almost twenty-fold higher affinity than the synthetic pentasaccharide control.

Furthermore, octasaccharide 6 binds to ATIII with higher affinity than a closely related enoxaparin octasaccharide (ΔIIa-IIs-Is-Is) described in Citizen Petition Supplement No. 1. The only difference between those two polysaccharides is the presence of a second 3-O-sulfo group in octasaccharide 6. Once again, a small difference in the structure of two polysaccharides of the same chain length leads to a difference in ATIII-binding affinity.

These findings further underscore the need to fully characterize all of enoxaparin’s polysaccharides. A method of characterization that did not account for each unique polysaccharide sequence would likely fail to identify structural differences leading to different biological activities, such as are found in the octasaccharides (for example) discussed above. Because these differences are expected to influence enoxaparin’s overall ATIII-binding properties, even in the absence of the classical ATIII-binding sequence, a generic product based on that incomplete characterization would likely have a different safety and efficacy profile than enoxaparin.

Furthermore, the published technique discussed above (see Figure 1) would not be able to fill this gap in information. That technique, for example, would not be able to distinguish between ΔIIa-IIs-Is-Is, which contains the classical ATIII-binding sequence, and octasaccharide 6, which does not. That is because the additional 3-O-sulfo group in octasaccharide 6 occurs in the disaccharide unit immediately adjacent to but not part of the tetrasaccharide surrogate (see Figure 1 above; the two boxes marked with a question mark).

Even if a new, previously unpublished technique were able to accurately measure the classical ATIII-binding sequence content, that would be an inadequate substitute for complete characterization. Dr. Boudier’s data in Table 2 make clear that one cannot assume that two LMWHs with the same classical ATIII-binding sequence content will have the same antithrombotic activity. Thus, even such an accurate measurement is inadequate because it cannot account for polysaccharides that do not contain that classical ATIII-binding sequence, but nonetheless bind to ATIII with high affinity. Moreover there are a number of other factors, wholly or partially independent of ATIII-binding, that influence enoxaparin’s antithrombotic activity (such as enoxaparin’s effect on TFPI). Nor can ATIII binding explain enoxaparin’s

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1 See Citizen Petition Supplement No. 1, at 6. $K_D = 34.1 \text{nM (SE = 58\% ; 1st series)}$; $K_D = 13 \text{nM (SE = 70\% ; 2nd series)}$.

2 Interestingly, such an increase in affinity has been predicted by model studies of synthetic variants of the classical ATIII-binding sequence. See van Beockel C and Petitou M. The unique antithrombin III binding domain of heparin: A lead to new synthetic antithrombotics. *Angew. Chem. Int. Ed. Engl.* 1993; 32:1671-1690.

3 Even the antithrombotic activities of enoxaparin that are ATIII-mediated also depend on several other factors. These include (1) the bioavailability of ATIII-binding polysaccharides (how effectively they reach ATIII in the bloodstream), (2) the pharmacokinetics of ATIII-binding polysaccharides (how effectively they are cleared from the bloodstream), (3) the sensitivity of ATIII-binding polysaccharides to the neutralizing action of platelet factor 4 released by activated platelets, and (4) the concentration of enoxaparin-binding plasma proteins in the bloodstream. Furthermore, the rate of clearance of polysaccharide chains in enoxaparin is complicated, involving such factors as the interaction of those polysaccharide chains with endothelial cells and plasma proteins. Certain of those factors (continued on next page)
inhibitory effects on inflammation, because these effects do not involve ATIII. Thus such a generic product could not be guaranteed to have the same safety and efficacy profile as enoxaparin in terms of bleeding complications and heparin-induced thrombocytopenia in patients.

The data presented above make clear that to structurally characterize enoxaparin one must structurally characterize each of enoxaparin’s polysaccharide chains. Any method of characterization that fell short of this requirement would likely fail to identify important differences within polysaccharides that make significant contributions to enoxaparin’s overall biological activities. As Dr. Boudier’s data demonstrate, some of these differences could affect clinically significant parameters such as antithrombotic activity.

C. Essentially Pure Polysaccharides Are Required for Structural Characterization

Structural characterization of enoxaparin requires determining the sequence of (i.e. “sequencing”) enoxaparin’s myriad different polysaccharides. The key step in the overall sequencing process is first obtaining a sufficiently pure sample of the polysaccharide to be sequenced. For example, to obtain the sequence of one of the over one-hundred 12mers in enoxaparin, one would first obtain a pure or essentially pure sample of that specific 12mer. One would then break that pure 12mer down into smaller fragments, analyze the structure of those smaller fragments, and reconstruct how those fragments fit together in the original 12mer. Separation and purification are required because, unlike proteins and DNA, heparin-derived polysaccharide sequences do not have one single, uniform underlying sequence.

That fragmentation and reconstruction process can only be performed with pure, or essentially pure, samples. If one were to break down a mixture of, for example, the over one-hundred different enoxaparin 12mers, one could not unambiguously assign the fragments back to their original parent polysaccharides using currently known procedures. Thus, without starting from a pure, or essentially pure sample, essential information about the sequences of the original polysaccharides could not be obtained. Accordingly, one could not link those fragments with the biological and clinical properties of the mixture.

(continued from previous page)


1 An essentially pure sample may, for instance, contain a major product and one minor impurity. See, e.g., Shriver Z, et al. Sequencing of 3-O-sulfate containing heparin deacetylglucuronic acid with a partial antithrombin III binding site. Proc. Natl. Acad. Sci. USA 2000; 97(19):10359-10364. See also Vives RR, et al. Sequence analysis of heparan sulphate and heparin polysaccharides. Biochem. J. 1999; 339:767-773. In contrast, enoxaparin is a highly complex mixture of polysaccharides, wherein, for example, there are over one-hundred different 12mers, as explained in the text.

1 If a polysaccharide is small enough, it may be possible to sequence it without breaking it into smaller fragments. Longer chains usually must be broken down.
D. Enoxaparin’s Longer Polysaccharides Have Not Been Adequately Purified Using Known Techniques

So far as sanofi-aventis is aware, the mixtures of longer polysaccharides found in enoxaparin have not been adequately purified to permit unambiguous sequencing using known techniques. That is because the ability of known techniques to separate enoxaparin polysaccharides from each other diminishes sharply as the length of those polysaccharides increases. Specifically, the bases for separation (e.g., differences in size, charge, and polarity) become incrementally smaller as polysaccharide length increases, thus complicating effective separation of longer polysaccharides.

As a result, longer enoxaparin polysaccharides, such as 12mers or greater, which have a molecular weight of about 3,600 Da or greater, are highly resistant to separation. There is no known publication reporting the complete separation of over one hundred 12mers, such as are present in enoxaparin. Moreover, there is no known publication reporting the separation of complex mixtures of yet longer polysaccharides, such as 18mers or greater, in which enoxaparin’s anti-Factor IIa activity resides, and which have a molecular weight of about 5,400 Da or greater.

This fact is extremely relevant to enoxaparin because enoxaparin contains a large number of these longer polysaccharide chains. Enoxaparin’s mass-average molecular weight is between 3,500 and 5,500 Da, and enoxaparin includes polysaccharides up to and exceeding 8,000 Da (i.e., 26mers). Moreover, as illustrated previously, the number of unique polysaccharide sequences increases exponentially with polysaccharide chain length. Accordingly, enoxaparin contains many more different 10mer sequences than 6mer sequences, and many more different 18mer sequences than 12mer sequences, for example. The exponentially larger number of different polysaccharide species with increasing chain length further complicates the separation of those longer species from each other. It is thus readily apparent why no publication has ever reported separating all of the longer enoxaparin polysaccharide species, such as 12mers and 18mers.

In general, heparin-derived polysaccharides may be separated on the basis of size, charge, and polarity. Chromatographic methods (e.g., size exclusion chromatography (SEC), anion exchange chromatography such as cetyltrimethylammonium strong anion exchange (CTA-SAX) chromatography), and reverse-phase high performance liquid chromatography (RP-HPLC) are frequently employed. Electrophoretic methods (e.g., polyacrylamide gel electrophoresis (PAGE) and capillary electrophoresis (CE)) are also frequently employed. However, these techniques have not been effectively used to separate enoxaparin’s longer polysaccharide chains from each other.

Moreover, the limitations of the spectroscopic detection methods chosen for observing such high molecular weight polysaccharides can further confound the difficulties of separation and isolation. For that reason, a spectroscopic detection may not necessarily reveal all polysaccharide species present in a sample.

A molecular weight of 3,600 Da corresponds approximately to a dodecasaccharide (12mer), employing a rough approximation of 300 Da/monosaccharide. In reality, the molecular weight of an average monosaccharide in an LMWH depends on the manufacturing process.

Characterization of enoxaparin is further complicated by the fact that sanofi-aventis’ manufacturing process creates both odd and even numbered polysaccharide chains. See Citizen Petition, at 12.
Characterizing Purified Polysaccharides Is a Laborious and Complicated Task, Even Using Sophisticated, Known Methods

Once purified, sequencing enoxaparin’s longer polysaccharides would remain a daunting challenge. Indeed, very few sequence determinations of longer heparin-derived polysaccharides have ever been published. To our knowledge, two relatively simple 14mers are the longest heparin-derived polysaccharides whose complete structures have been reported. And those structures were reported more than ten years ago, in 1995.

Even the structural characterization of the shorter enoxaparin polysaccharides, which theoretically can be separated and sequenced, is anything but routine. Sequencing polysaccharides is difficult, laborious, and time-consuming, because it usually involves sequential application of a variety of different analytical techniques.

Sequencing involves first separating and purifying the individual polysaccharides by multiple chromatographic and/or electrophoretic steps, followed by characterizing each purified polysaccharide by analytical techniques such as mass spectrometry, enzymatic and/or chemical fragmentation, or nuclear magnetic resonance (NMR). In most cases, it is necessary to interpret each analytical result before selecting the most appropriate analysis to conduct next. Often, the set of methods required for characterization varies with the specific polysaccharide being analyzed, as different sequences present different challenges. While computer programs may help to analyze the data, they cannot replace or even speed the pace of those analytical experiments.

The non-routine nature of such sequencing can be demonstrated by reference to the scientific literature. For example, sequencing a particular heparin decasaccharide (10mer) entailed a stepwise, iterative strategy involving: determining the mass of the entire 10mer by mass spectrometry; identifying the individual disaccharide building blocks of the 10mer after exhaustive enzymatic degradation; fragmenting the 10mer by two different methods; analyzing the resulting, individual fragments by mass spectrometry and electrophoresis; piecing the 10mer sequence together from the various fragments; and finally confirming the sequence assignment with NMR. Although a computer program was used to assist in converging on the polysaccharide sequence, sequencing that 10mer still required performing a number of different analytical methods on what was essentially a pure sample.

Moreover, sanofi-aventis knows from experience just how laborious and difficult it is to characterize polysaccharides. Sanofi-aventis has spent many person-years analyzing the chemical composition of enoxaparin. Despite significant effort, the process is so laborious that, to date, sanofi-aventis has been able to sequence only a small fraction of the enoxaparin polysaccharides that can theoretically be separated. In fact, the overwhelming majority of enoxaparin polysaccharides remain unidentifed.

Comparison of Traditional Measurements and Other Known Structural Characteristics Is Inadequate to Establish Sameness

Because so much of enoxaparin remains uncharacterized at present, ANDA applicants may attempt to demonstrate sameness of active ingredient by comparing traditional
LMWH measurements as well as known structural characteristics. However, even if a generic manufacturer's product were similar to enoxaparin in terms of one or more traditional measurements and/or known structural characteristics, that product would not necessarily have the same clinical activity and safety profile as enoxaparin.

Such an approach is inadequate because even taken together, these features account for only some of enoxaparin's many biological and clinical properties described above. They thus do not take into account the contributions of other structural features of enoxaparin that contribute to enoxaparin's overall biological and clinical effects. Indeed, because so much remains unknown about the mechanisms that cause enoxaparin's various clinical effects, several of enoxaparin's clinical actions have not yet been linked to any known structural feature in the polysaccharide mixture. As a result, until enoxaparin is fully characterized, the only way to establish sameness is to use a manufacturing process that is equivalent to sanofi-aventis' process. Otherwise, the generic application must be supported by clinical trials to establish safety and efficacy.

An example of an additional, known structural feature found in enoxaparin is a 1,6 anhydro ring structure located at one end of about 15-25% of the polysaccharide chains in the enoxaparin mixture. In the Citizen Petition and Supplements, sanofi-aventis demonstrated that the 1,6 anhydro ring structure influences a number of enoxaparin's biological properties. Therefore, a generic product that does not contain the same (15-25%) 1,6 anhydro ring structure content as enoxaparin cannot be the same as enoxaparin. However, although the 1,6 anhydro ring structure influences many of enoxaparin's biological properties, recent studies conducted by sanofi-aventis demonstrate that other process-dependent structural features also influence many of these same activities. As a result, although having the same 1,6 anhydro ring structure content as enoxaparin is necessary for a demonstration of sameness, it is not sufficient.

For example, sanofi-aventis has discovered that although the 1,6 anhydro ring structure influences the capacity of enoxaparin to enhance the rate of Factor VIIa inhibition by ATIII, other process-dependent structural features of enoxaparin also influence this biological activity. Factor VIIa is a clinically important blood coagulation factor, regulated in part by ATIII. Factor VIIa initiates coagulation when complexed to tissue factor. Abnormally high blood levels of Factor VIIa have been reported in patients with myocardial infarction and unstable angina, two of enoxaparin's FDA-approved indications. Enoxaparin has been found to lower those high blood levels of Factor VIIa in patients with unstable angina. Therefore, Factor VIIa inhibition is an important indicium of enoxaparin's clinical activity in treating arterial thrombosis patients.

These studies analyzed the properties of fifteen samples described in Appendix C, including enoxaparin, the two previously described LMWHs with different 1,6 anhydro ring structure content, and fractions and polysaccharides isolated therefrom. Again, as shown in Appendix C, about 15-25% of polysaccharides in enoxaparin contain a 1,6 anhydro ring structure. (See sample 9 in Appendix C.) The two other LMWHs were made by a similar, but non-equivalent, process to that for making enoxaparin, and have similar molecular weights, anti-Xa activities, and anti-IIa activities as enoxaparin. See Citizen Petition, at 13-15. Although the possible existence of other differences cannot be excluded, they differ from enoxaparin by having lower or higher 1,6 anhydro ring structure content: < 7% or 40-50%, respectively. See samples 10-11 in Appendix C.
In the study reported in Appendix D (see Appendix C for a description of the samples), Professor M. Samama of the Hôtel Dieu Hospital in France measured the ability of each of fifteen samples to catalyze the inhibition of Factor VIIa by ATIII. Among the samples tested were enoxaparin (containing 15-25% 1,6 anhydro ring structure content), an LMWH containing < 7% 1,6 anhydro ring structure content (“<7% 1,6 anhydro LMWH”) and an LMWH containing 40-50% 1,6 anhydro ring structure content (“40-50% 1,6 anhydro LMWH”). Professor Samama determined that those three different LMWHs exhibited different minimum concentrations needed to catalyze the inhibition of Factor VIIa by ATIII. Hence, enoxaparin’s ability to catalyze the inhibition of Factor VIIa by ATIII is, to some extent, related to its 1,6 anhydro ring structure content.

However, Professor Samama also analyzed three pure enoxaparin octasaccharides that do not contain the 1,6-anhydro ring structure. Professor Samama found that the minimum concentration that catalyzed the inhibition of Factor VIIa by ATIII varied significantly among those three octasaccharides as well. Thus, other process-dependent structures in addition to the 1,6 anhydro ring structure must also affect enoxaparin’s capacity to catalyze Factor VIIa inhibition by ATIII. In each of the three pure octasaccharides, the classical ATIII-binding sequence is present within a different flanking sequence context. As in Dr. Boudier’s experiments above, the different sequences flanking the classical ATIII-binding sequence may result in variations in the ability to enhance the inhibition of Factor VIIa.

A second study was conducted examining one of enoxaparin’s anti-inflammatory activities: inhibition of the contact-kinin system. That study demonstrates that although the same 1,6 anhydro ring structure content influences enoxaparin’s effect on contact-kinin system inhibition, other factors also shape enoxaparin’s effect. The contact-kinin system, one of enoxaparin’s anti-inflammatory targets, is a complex collection of proteins that link coagulation with inflammation. When the contact-kinin system is activated, another protein system called the complement system in turn becomes active, leading to inflammation. That inflammation is thought to be at the root of arterial thrombosis. Enoxaparin inhibits the activation of the contact-kinin system in vitro.

Professor L. Bergamaschini of Maggiore Hospital at the University of Milan in Italy conducted a series of studies on inhibition of beta-amyloid-induced activation of HK by each of the fifteen samples described in Appendix C. As reported in Appendix E, Professor Bergamaschini’s data suggest that enoxaparin inhibits the activation of high molecular weight kininogen (HK), and thus inhibits the contact-kinin system, somewhat more than does the <7% 1,6 anhydro LMWH and somewhat less than does the 40-50% 1,6 anhydro ring structure content. Hence, the extent of enoxaparin’s inhibition of contact-kinin system activation is somewhat correlated with its 1,6 anhydro ring structure content. Therefore, a generic product that does not contain the same 1,6 anhydro ring structure content as enoxaparin may not affect the contact-kinin system to the same degree as enoxaparin, and thus may have different anti-inflammatory effects in patients.

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9 The three tested octasaccharides each have different sets of monosaccharide residues flanking the classical ATIII-binding sequence.
However, Professor Bergamaschini’s data also demonstrate variability between individual fractions in terms of inhibition of the contact-kinin system. (See Appendices C and E.) For example, although the studied 6mer, 8mer, and 12mer fractions from enoxaparin appear less inhibitory than the corresponding fractions from the < 7% 1,6 anhydro LMWH, the studied enoxaparin 10mer fraction resulted in greater inhibition than did the 10mer fraction of the < 7% 1,6 anhydro LMWH. Furthermore, when comparing the studied enoxaparin fractions against each other, the 10mer fraction of enoxaparin was more active than the others. Because the various fractions from each LMWH have about the same overall 1,6 anhydro ring structure content, those results suggest that additional, unknown, but process-dependent structural features within the enoxaparin 10mer fraction also contribute to enoxaparin’s inhibition of contact-kinin system activation.

As a result of these experiments, plus data previously presented in the Citizen Petition and Supplements, it is clear that enoxaparin’s 1,6 anhydro ring structure content (15-25%) influences many of enoxaparin’s important biological activities. Thus, any proposed generic product that does not contain the same range of 1,6 anhydro ring structure content as enoxaparin may not have the same clinical effects as enoxaparin, and therefore cannot be considered to be the same. However, Professor Samama’s and Professor Bergamaschini’s data also demonstrate that other factors, independent of the 1,6 anhydro ring structure, also influence Factor VIIa and contact-kinin related activities. Thus, even a generic product that has the same 1,6 anhydro ring structure content as enoxaparin is not necessarily the same as enoxaparin.

Similarly, any attempt to establish sameness by showing that the relative proportions of component disaccharides and other small oligosaccharides (i.e. “building blocks”) in a proposed generic product are equivalent to those found in enoxaparin is also inherently flawed. It is not the relative amount of particular building blocks in an LMWH that endows that LMWH with its unique biological activities. Instead, it is the different sequences in which those building blocks are arranged from one end of each polysaccharide chain to the other that endow an LMWH with its overall biological properties. Merely determining how much of each type of building block is present in a complex mixture thus provides no information about the sequences of the LMWH polysaccharide mixture and, accordingly, no information about the biological and clinical activities of that mixture.

IV. Conclusion

Enoxaparin is a complex mixture of polysaccharide molecules and has many different biological activities. Moreover, the identities of the enoxaparin polysaccharides that mediate several of those biological activities and their related clinical effects are poorly understood or in some cases unknown. Therefore, even establishing that a generic product contains all of the same currently known structural characteristics as enoxaparin is not sufficient to establish sameness.

For example, such a building block analysis, when performed on a complex mixture of polysaccharides, is akin to attempting to determine the meaning of a page of text by determining the total number of each of the 26 letters of the alphabet the text contains. It is the arrangement of the letters into words, not the type and number of the individual letters that are present, that provides meaning.
In order to fully characterize enoxaparin, one must sequence each of enoxaparin’s unique polysaccharide chains. Until such full characterization of enoxaparin is accomplished, the only way for a proposed generic to establish that it has the same mixture of different active ingredients as enoxaparin is to use a manufacturing process that is demonstrated to be equivalent to sanofi-aventis’ manufacturing process. Absent using an equivalent manufacturing process, a proposed generic would have to demonstrate proof of equivalent safety and effectiveness through clinical trials.
CERTIFICATION

The undersigned certifies that, to the best knowledge and belief of the undersigned, this Supplement includes all information and views on which the Supplement relies, and that it includes representative data and information known to the petitioner which are unfavorable to the petition.

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1 See Citizen Petition, at 1.

4 See Citizen Petition, at 10, and 15-16.

5 See id. at 6.

6 See Citizen Petition Supplement No. 1, at 4-7. See also data presented in Section II(B) of this paper.

7 See, e.g., Citizen Petition Supplement No. 1, at 4-7.


9 See Appendix B.


12 See Citizen Petition, at 6.

13 See Citizen Petition, at 19.


(continued from previous page)


22 See id., and see Citizen Petition, at 1; under “actions requested.”


26 See id.

27 See Appendices C and D.

28 See id.


33 See Appendix E.

34 See id.

35 See id.
