Contaminants in the Recalled Unfractionated Heparin Preparations:
Pharmacologic and Clinical Implications

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September 2008

Disclosures

Nothing to disclose

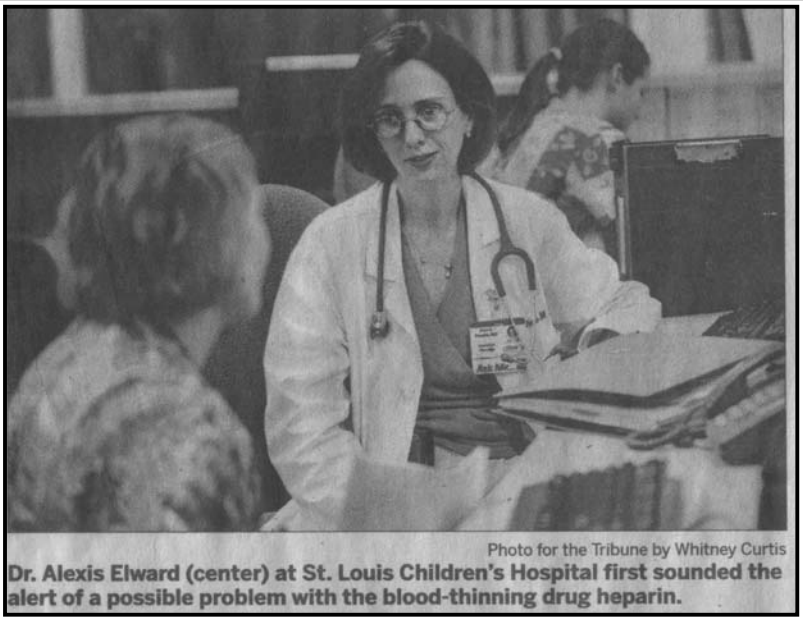


Photo for the Tribune by Whitney Curtis

Dr. Alexis Elward (center) at St. Louis Children's Hospital first sounded the alert of a possible problem with the blood-thinning drug heparin.

Dr. Edward first reported to CDC the serious and unusual adverse events in pediatric patients treated with heparin (November 2007).

INTRODUCTION

- Jan. 1, 2007 to May 31, 2008: Over 246 deaths reported to the FDA associated with heparin with 238 of these reported on or after Jan. 1, 2008
  - causes of death?
  - heparin related deaths?
- 149 deaths (146 reported on or after Jan. 1, 2008) fit the profile of allergic reactions or hypotension / anaphylaxis
  - concurrent drug therapy?
  - delayed immunogenic responses
  - pathologic predisposition

FDA Jun 30, 2008

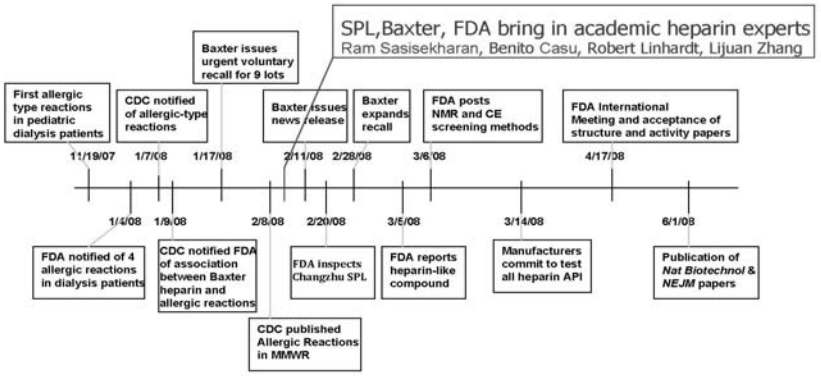
Potential Sources of a Heparin Contaminant

1. Substandard raw material
2. Carryover during production
3. Mixing of batches
4. Mixing of heparin from different species
5. Preservatives or other additives
6. Intentional approaches

Heparin Raw Material

- 2/3 of the world's supply of hog mucosa originates from China; most of the remaining 1/3 is produced in North America and the European Community
- The quantity of raw material varies depending on the origin
- Until March 2008 there were no NMR and CE requirements for acceptance of heparin in the US

Timeline



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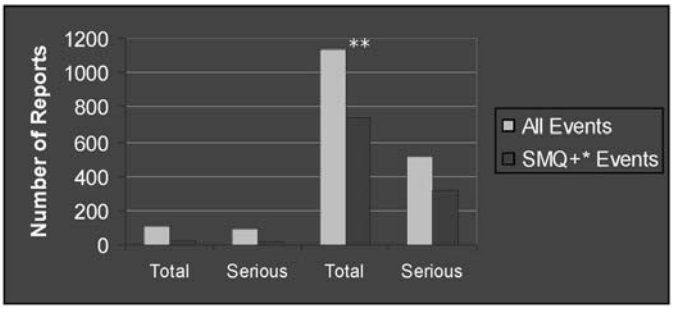
Adverse Reactions



*SMQ+ means events that included one or more allergic and/or hypotensive symptom(s)
** 1:10,000 incidence

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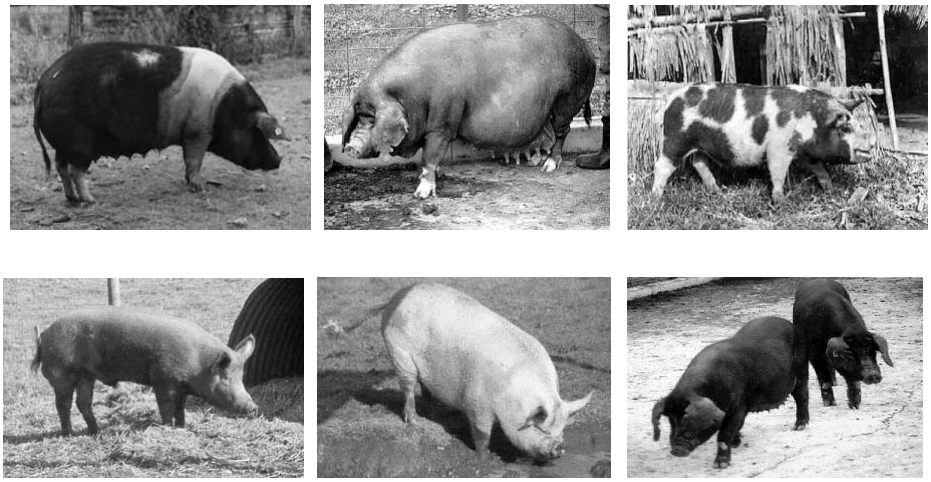
All Swine are Not the Same

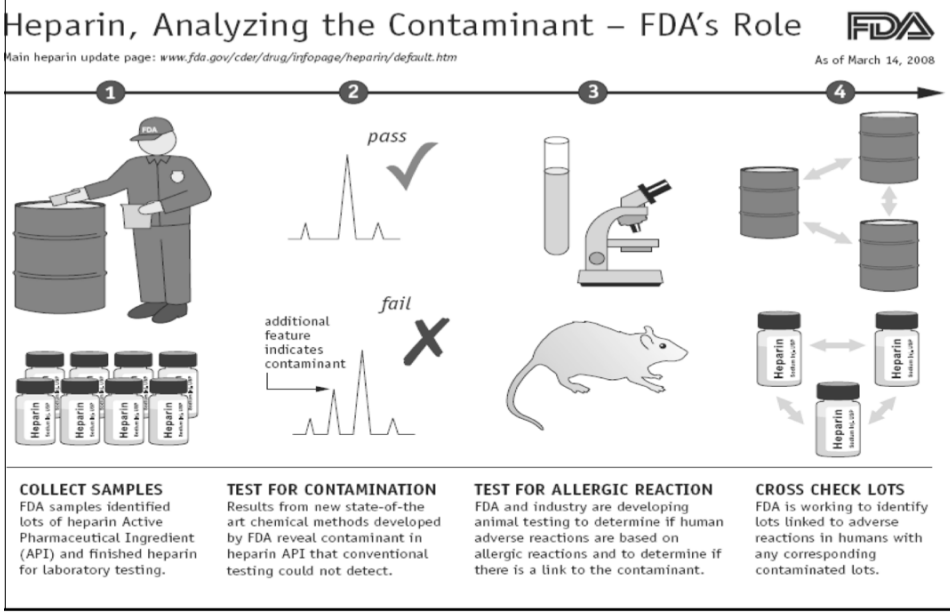


Variations in heparin composition are noted between species.

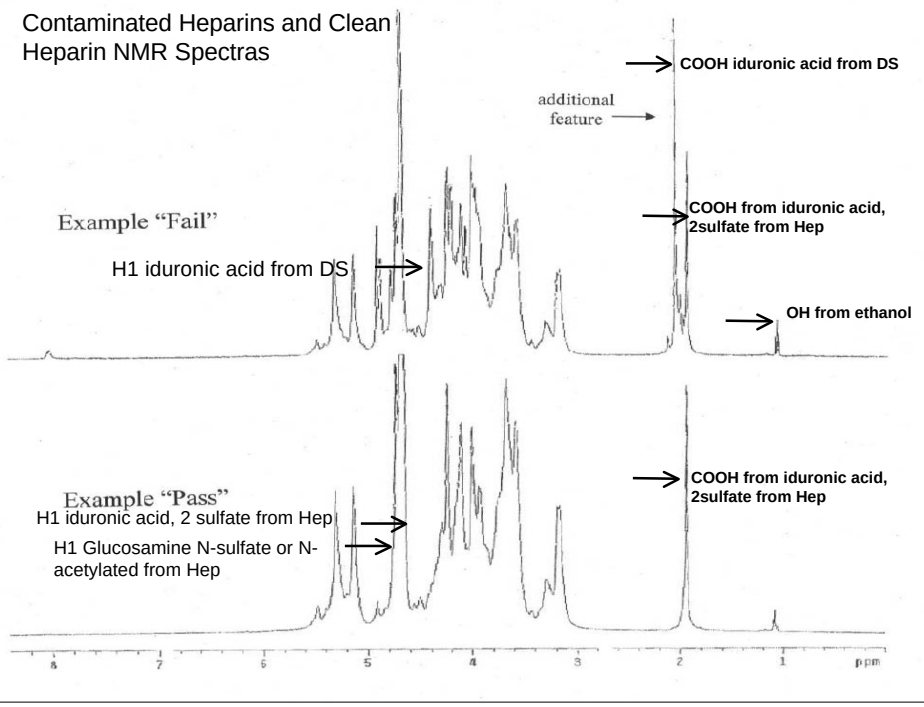
HEPARIN RECALL

- **The recalled batches of heparin were found to contain a novel over-sulfated chondroitin sulfate (OSCS) added to heparin**
- **The contaminant was present in varying amounts (5 - 30%)**
- **Some LMWHs were also found to contain the contaminant**

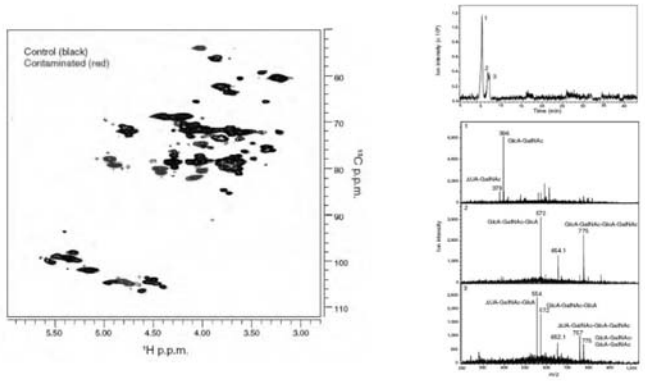
FDA's Position



Contaminated Heparins and Clean Heparin NMR Spectras

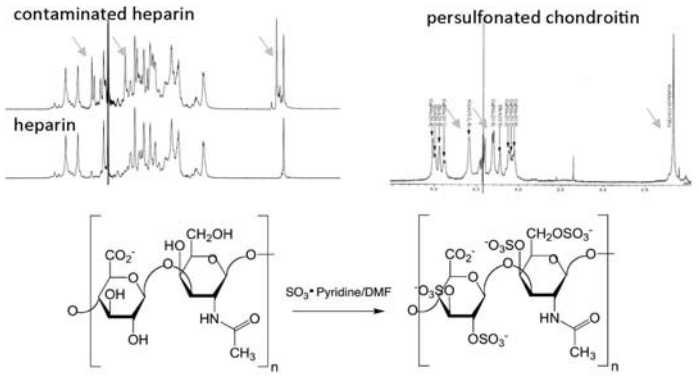


Contaminated heparin from China

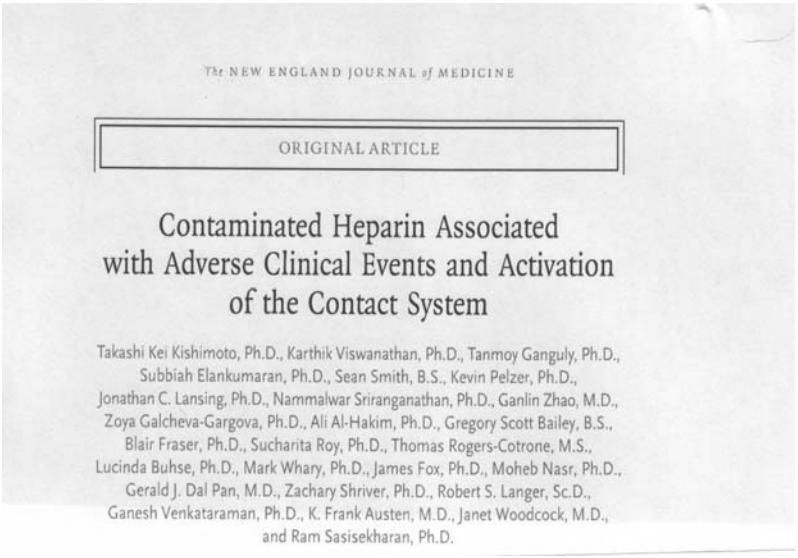


Guerrini, *et al.*, *Nature Biotechnology*, 26, 669. 2008

Contaminant found in heparin over sulfated chondroitin, OSCS



Guerrini, *et al.*, *Nature Biotechnology*, 26, 669. 2008
Maruyama, *et al.*, *Carbohydrate Research*, 306, 35, 1998



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Contaminated Heparin Associated with Adverse Clinical Events and Activation of the Contact System

Takashi Kei Kishimoto, Ph.D., Karthik Viswanathan, Ph.D., Tanmoy Ganguly, Ph.D., Subbiah Elankumaran, Ph.D., Sean Smith, B.S., Kevin Pelzer, Ph.D., Jonathan C. Lansing, Ph.D., Nammalwar Sriranganathan, Ph.D., Ganlin Zhao, M.D., Zoya Galcheva-Gargova, Ph.D., Ali Al-Hakim, Ph.D., Gregory Scott Bailey, B.S., Blair Fraser, Ph.D., Sucharita Roy, Ph.D., Thomas Rogers-Cotrone, M.S., Lucinda Buhse, Ph.D., Mark Whary, Ph.D., James Fox, Ph.D., Moheb Nasr, Ph.D., Gerald J. Dal Pan, M.D., Zachary Shriver, Ph.D., Robert S. Langer, Sc.D., Ganesh Venkataraman, Ph.D., K. Frank Austen, M.D., Janet Woodcock, M.D., and Ram Sasisekharan, Ph.D.

Manuscript focused on the activation of FXIIa leading to the formation of bradykinin as the main mechanism of the multiple adverse effects observed with contaminated heparin.

Janet Woodcock FDA

FDA Confirms Heparin Contaminant as Cause of Deaths

Published: April 21, 2008

Jin Shaohong, deputy director-general of China's National Institute for the Control of Pharmaceutical and Biological Products, a member of a Delegation of Chinese medical officials, gestures during a news conference at the Chinese Embassy in Washington, Monday, April 21, 2008 where he expressed doubts that a component from a factory in China was to blame for the problems with the blood thinner Heparin. (AP photo/J Scott Applewhite)

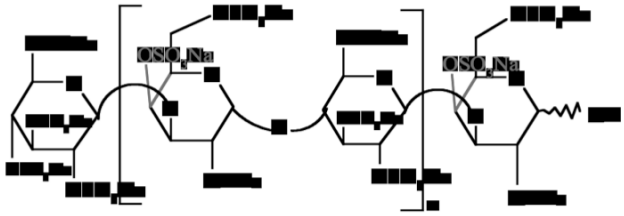
The Main Contaminant*
Over-Sulfated Chondroitin Sulfate

- **The contaminant was isolated from several recalled heparin preparations**
- **Hemi-synthetic OSCS was prepared from porcine cartilage and compared with the isolated heparin contaminant in chemical, biological and pharmacological assays**

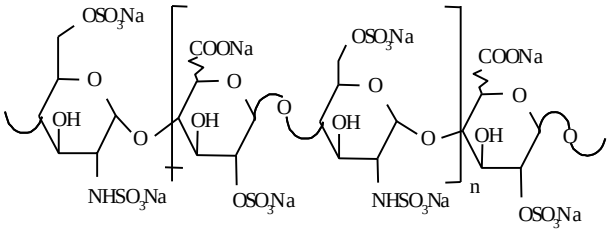
*** Other contaminants such as hyper-sulphated dermatans and heparans and marine GAGs may be present.**

*

Comparative Structures of Oversulfated Chondroitin Sulfate and Heparin

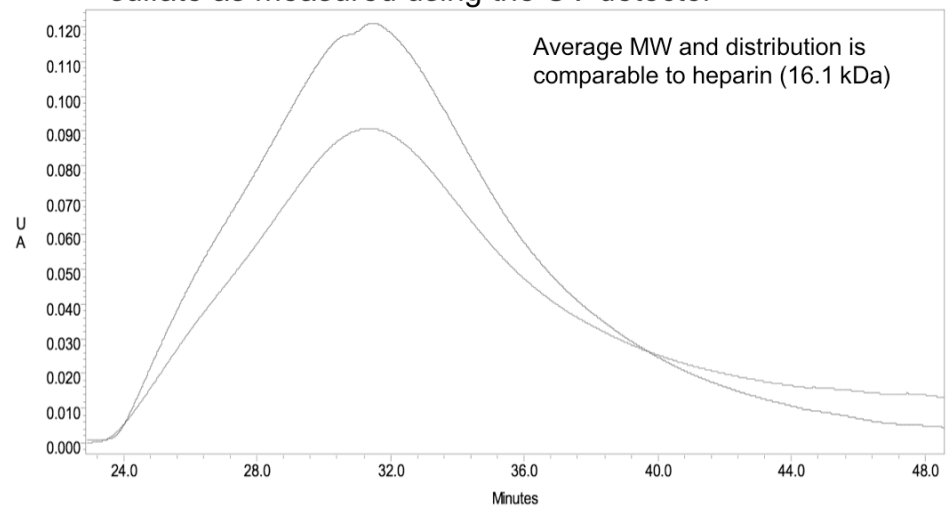


Oversulfated chondroitin sulfate



Heparin

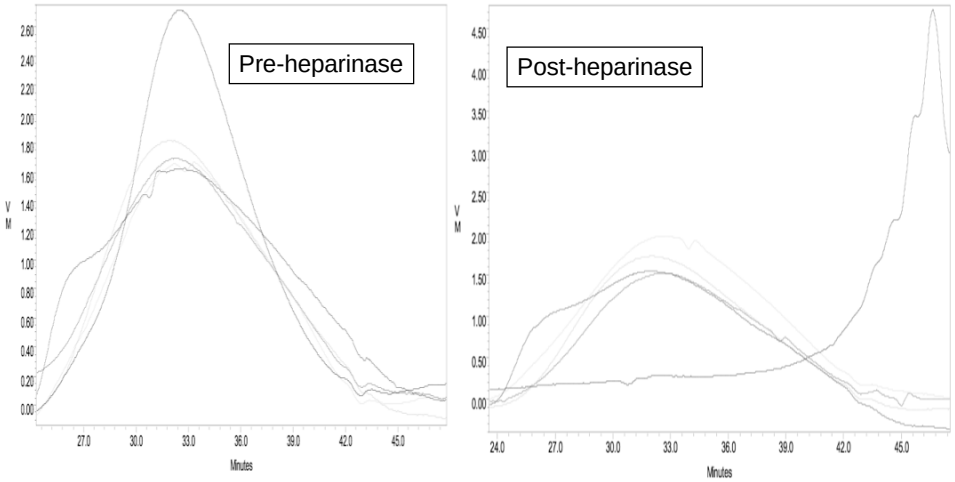
Heparinase-I digestion profile of oversulfated chondroitin sulfate as measured using the UV detector



Oversulfated chondroitin sulfate

Oversulfated chondroitin sulfate + heparinase-I

Heparinase-I digestion profile of uncontaminated heparin and contaminants isolated from recalled heparins as measured using the UV detector



Uncontaminated Heparin

Isolated Contaminant-2

Isolated Contaminant-4

Isolated Contaminant-1

Isolated Contaminant-3

Heparinase-1 Activity is Inhibited by Heparin Contaminants

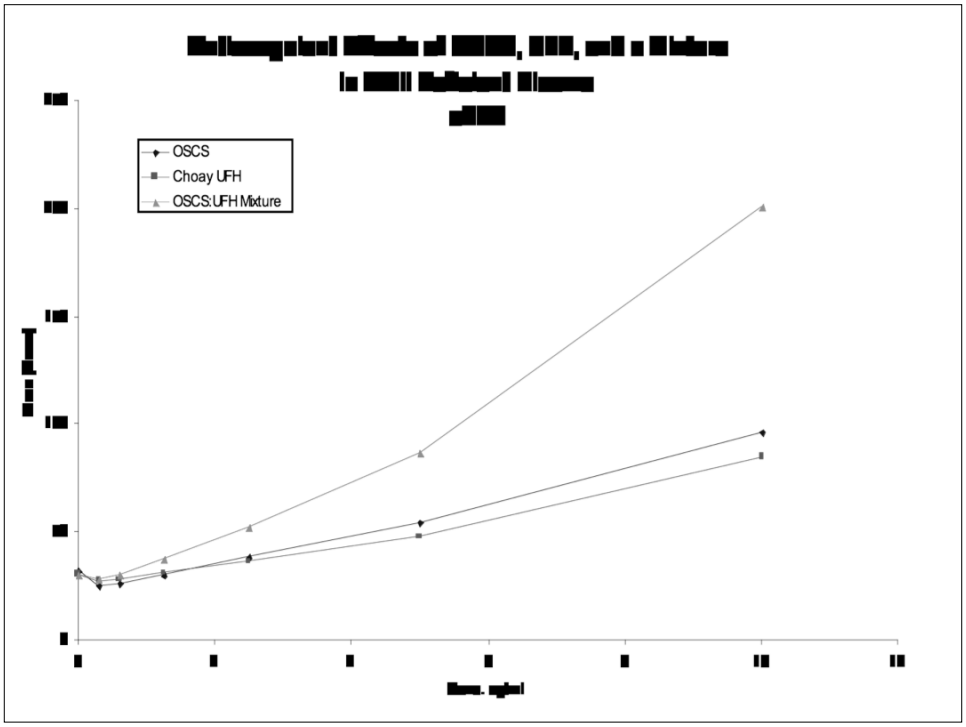
Agent	IC_{50}
HCS-1	0.24 μM
HCS-2	0.31 μM
HC	0.28 μM

Contaminant-free unfractionated heparin was used in these studies as a substrate. Added OSCS decreased the degradation of heparin by heparinase-1.

HCII and ATIII Affinity Profiles of Hypersulfated Chondroitin Sulfate and Heparin Contaminant

Agent	HCII	ATIII
HCS-1	32%	<5%
HCS-2	26%	<5%
Heparin	24%	28%
HC	26%	<5%

The heparin contaminant has no affinity to ATIII but has HCII mediated anticoagulant activity similar to that of UFH.



Anticoagulant and Anti-Protease Assay Results

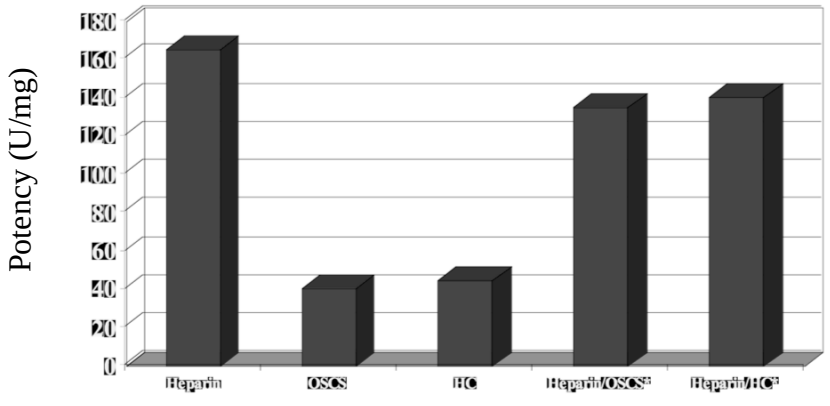
- The heparin contaminant has assay dependent anticoagulant activity in whole blood and plasma:
  - PT = no effect
  - aPTT, Heptest = 10-40 U/mg
  - Thrombin Time (clot-based) = 20-50 U/mg
  - Anti-IIa = ~25 U/mg
  - Anti-Xa = no effect

Neutralization of the Biological Properties of Heparin Contaminants

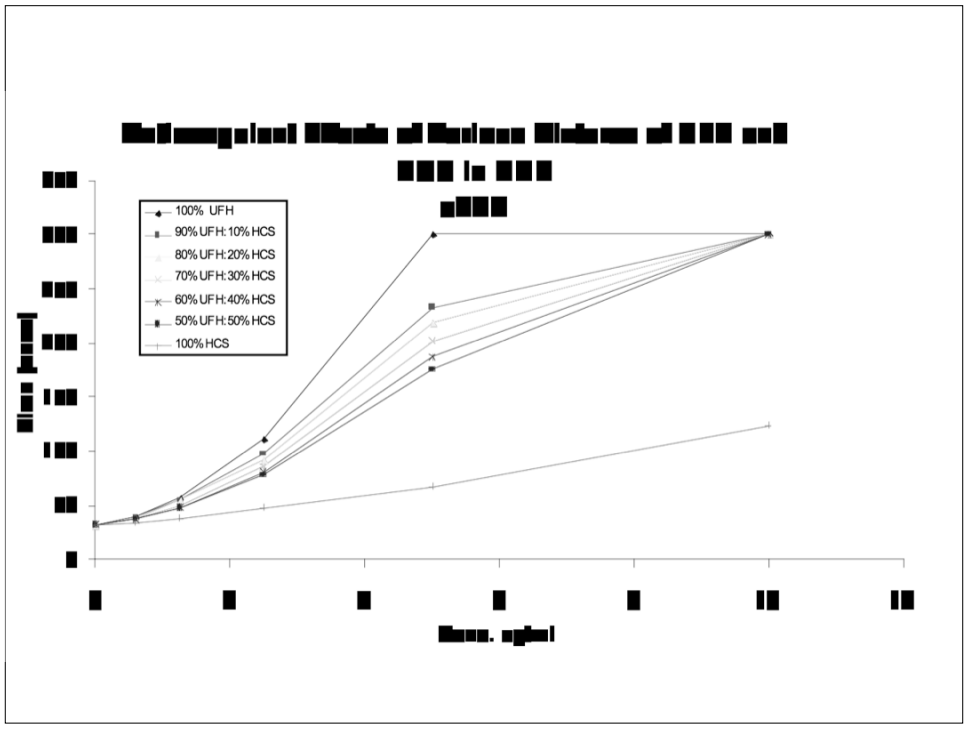
Agent	Protamine Neutralization	PF4 Neutralization	Polybrene Neutralization
HCS-1	+	+	+
HCS-2	+	+	+
Heparin	+	+	+
HC	+	+	+

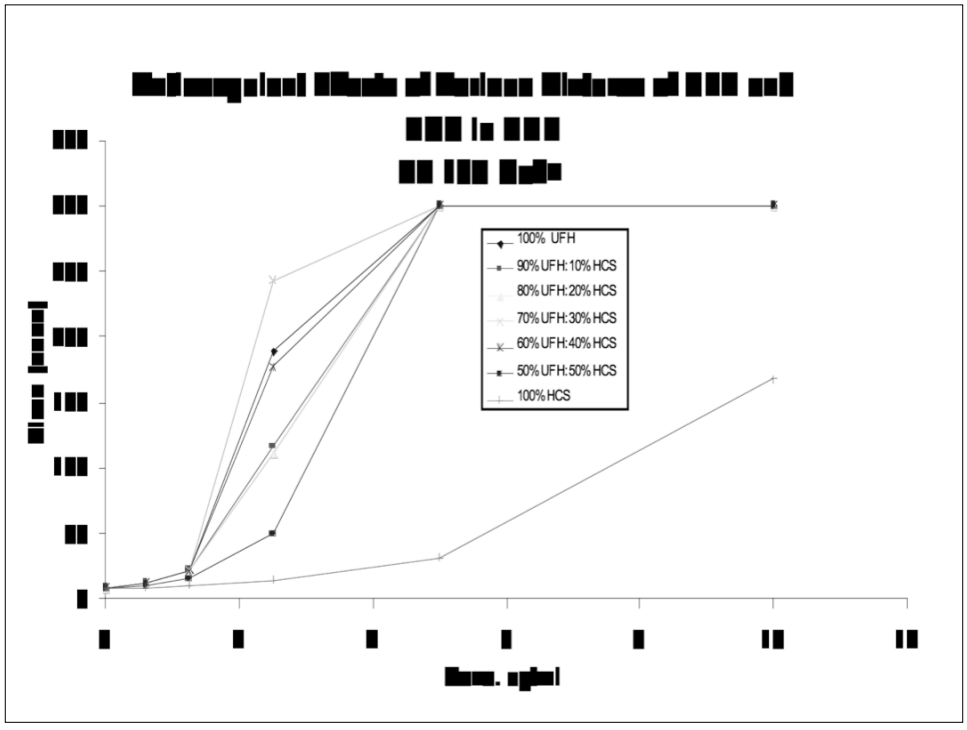
Tested by the aPTT, TT, anti-IIa assays.

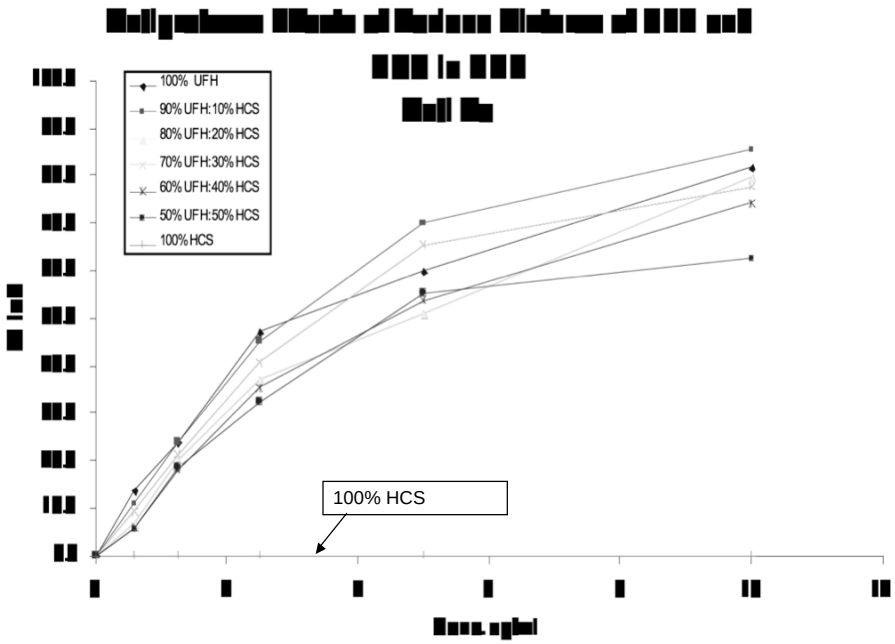
Effect of OSCS and HC on the Anticoagulant Activity of UFH



*1:1 mixture


100% UFH
90% UFH: 10% HCS
80% UFH: 20% HCS
70% UFH: 30% HCS
60% UFH: 40% HCS
50% UFH: 50% HCS
100% HCS


100% UFH
90% UFH: 10% HCS
80% UFH: 20% HCS
70% UFH: 30% HCS
60% UFH: 40% HCS
50% UFH: 50% HCS
100% HCS



Comparative Studies on the Antithrombotic Profile of CFH and Contaminated Heparin in Rats

Agent	# Laser Shots
Contaminant-free Heparin	4.8 ± 0.6
Contaminated Heparin	5.4 ± 0.9
Saline	2.6 ± 0.4

All agents were tested at 2 mg/kg iv.

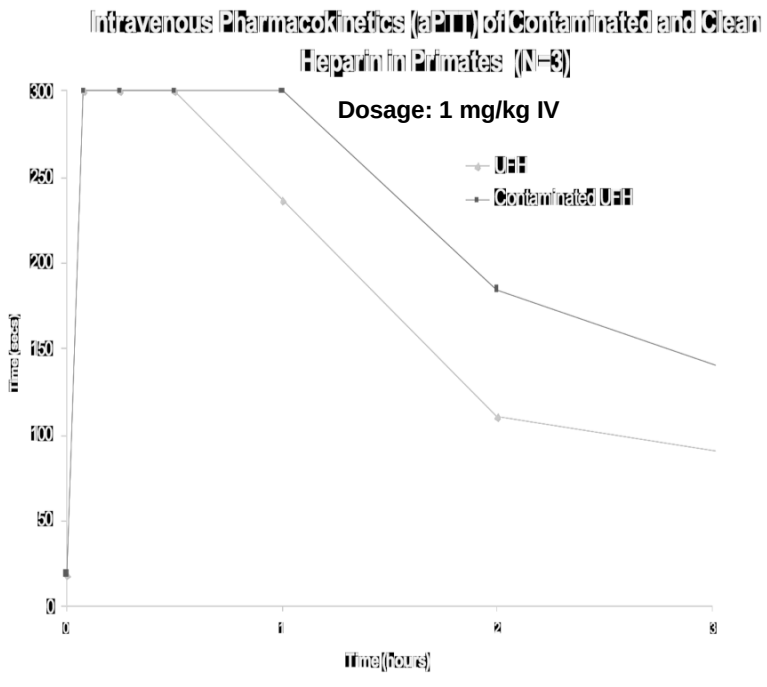
Contaminated heparin produced stronger antithrombotic effects.

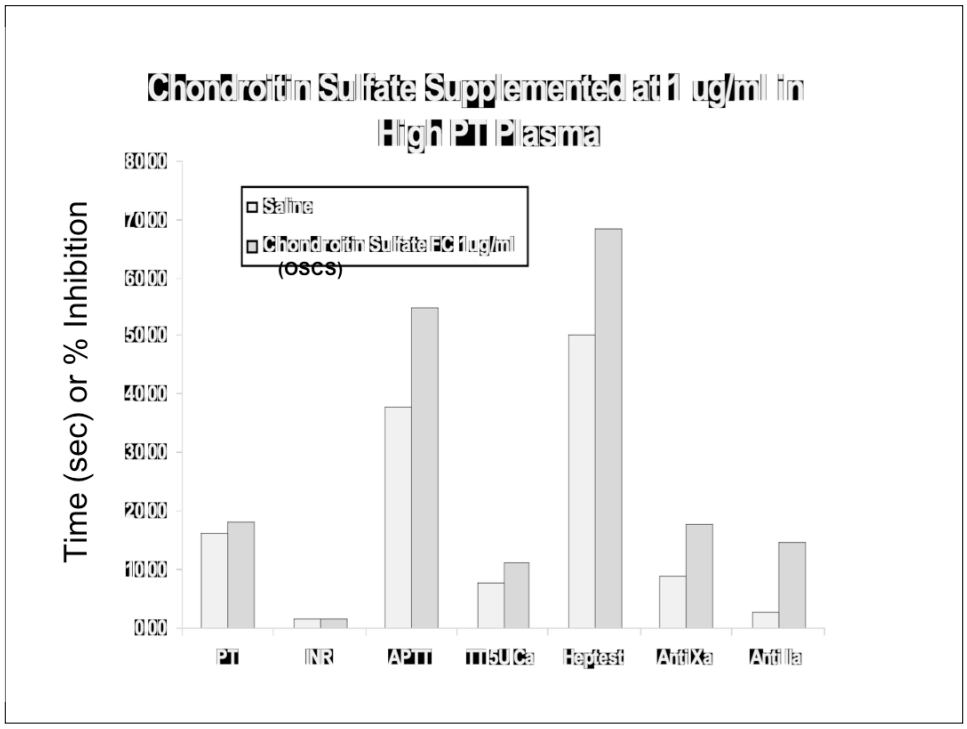
Comparative Studies on the Bleeding Profile of CFH and Contaminated Heparin in Rats

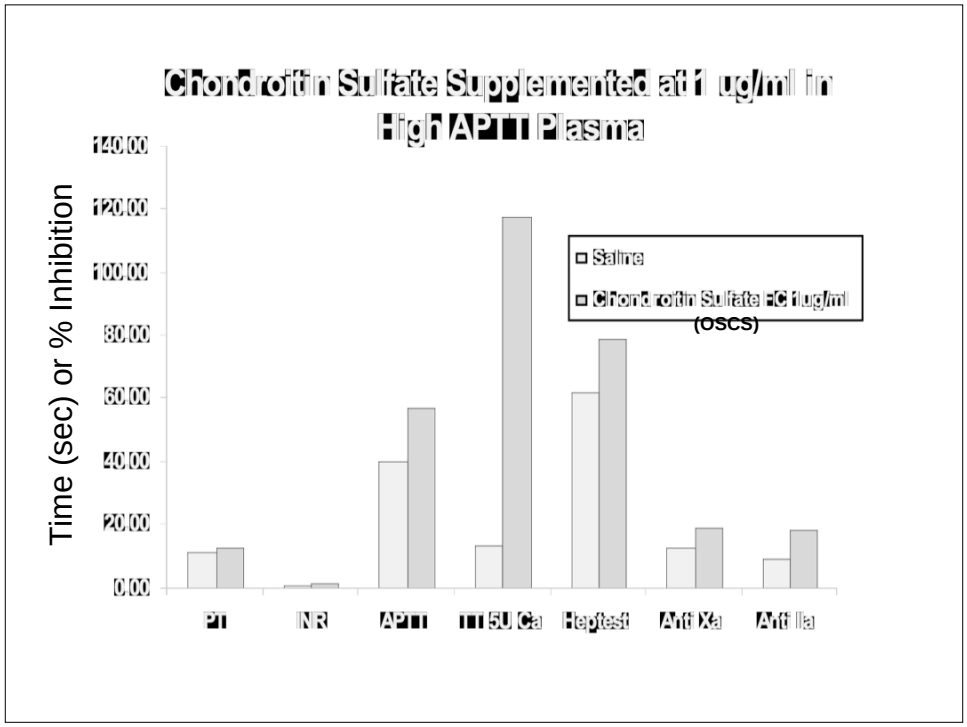
Agent	Bleeding Time
Contaminant-free Heparin	22.2 ± 4.6 min.
Contaminated Heparin	36.8 ± 6.1 min.
Saline	5.1 ± 2.6 min.

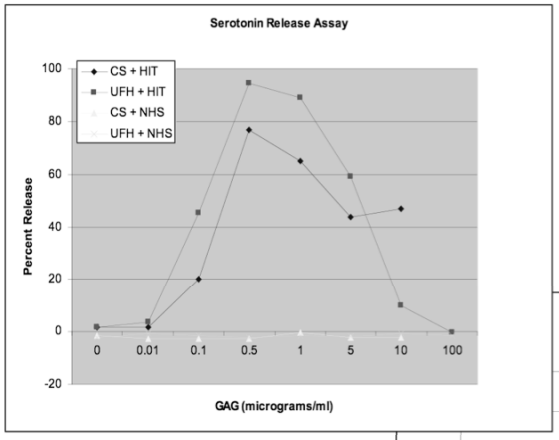
All agents were tested at 2 mg/kg iv.

Contaminated heparin produced stronger bleeding effects.

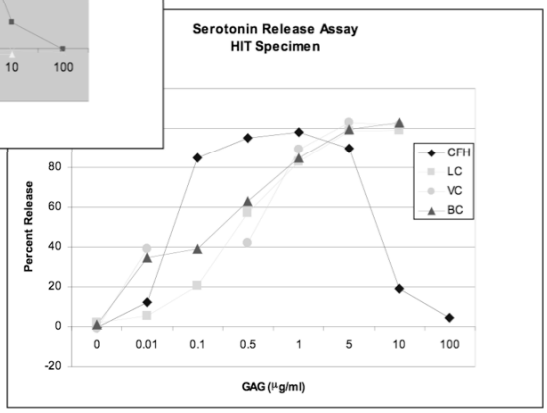



Chondroitin Sulfate Supplemented at 1 ug/ml in High PT Plasma
Time (sec) or % Inhibition
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Saline
Chondroitin Sulfate FC 1ug/ml (OSCS)
PT
INR
APTT
TT 5U Ca
Heptest
Anti Xa
Anti Ia

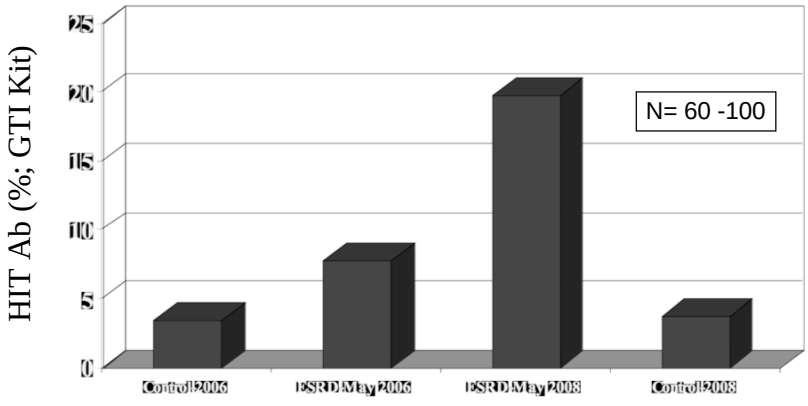

Chondroitin Sulfate Supplemented at 1 ug/ml in High APTT Plasma
Time (sec) or % Inhibition
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Saline
Chondroitin Sulfate FC 1ug/ml (OSCS)
PT
INR
APTT
TT 5U Ca
Heptest
Anti Xa
Anti Ia



Interaction of OSCS with HIT Antibodies



HIT Antibody Profiling in ESRD Patients on Maintenance Hemodialysis



Over-sulfated Chondroitin Sulfate Contamination in Heparins:
Biological Implications

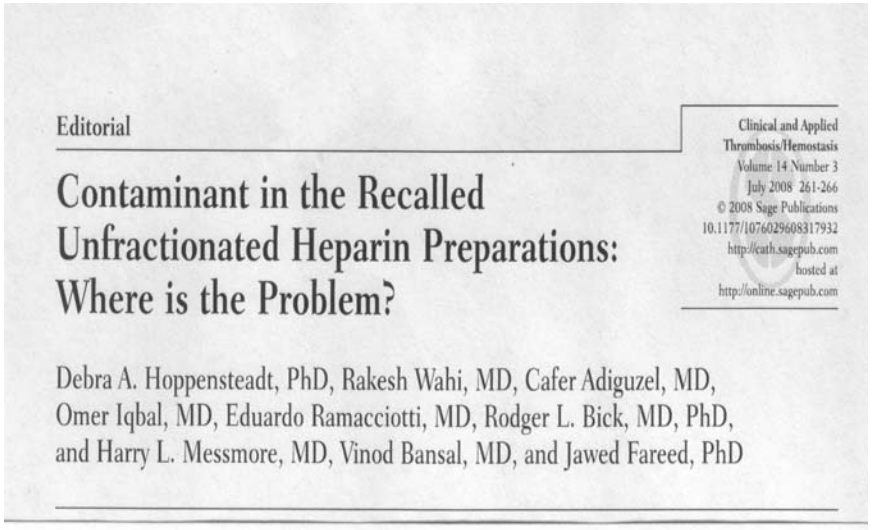
1. Varying biological interactions as measured by different assays
2. Prolonged anticoagulant action after iv administration
3. Pharmacodynamic enhancement due to the inhibition of heparin digesting enzymes
4. Increased immunogenic response
5. Increased binding to plasma proteins
6. Displacement of protein-bound heparin

Adverse Reactions to Contaminated Heparin May Not be Totally Related to Contact Activation

1. Reported adverse reactions to contaminated heparins are complex and multifactorial.
2. Reported reactions to contaminated heparins differ in different groups of patients.
3. Drug interactions with anti-hypertensive agents (ACE-I) may augment the SAE.
4. Repeated exposure to OSCS may result in the generation of antibodies to OSCS-PF4 (or other bound protein) resulting in an anaphylactic response.
5. Patient response variations may be due to antibody generation.
6. Retrospective antibody profiling should be done.

Clinical Implications

- **The presence of the contaminant may alter the pharmacokinetics and pharmacodynamics of heparins.**
- **The contaminant exhibits interactions with heparin which could impact the safety and efficacy of heparin therapy.**
- **The contaminant may be more immunogenic than heparin resulting in the generation of heterogeneous antibodies.**



Editorial

Contaminant in the Recalled Unfractionated Heparin Preparations: Where is the Problem?

Clinical and Applied Thrombosis/Hemostasis
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Debra A. Hoppensteadt, PhD, Rakesh Wahi, MD, Cafer Adiguzel, MD, Omer Iqbal, MD, Eduardo Ramacciotti, MD, Rodger L. Bick, MD, PhD, and Harry L. Messmore, MD, Vinod Bansal, MD, and Jawed Fareed, PhD

Editorial points to the complex nature of adverse reactions and deaths associated with contaminated heparin. Suggests the possibility of other contaminants in heparin and other factors responsible for reactions.

US heparin supply

- 2007
  - Baxter Healthcare Corporation ~45% heparin sales
  - American Pharmaceutical Partners ~45% heparin sales
  - Hospira ~10% of heparin sales
  - Sanofi-Aventis controlling share of low molecular weight heparin
- 2008 after Baxter February 28 recall
  - American Pharmaceutical Partners ~90%
  - Hospira ~10%
  - Sanofi-Aventis controlling share of low molecular weight heparin

Heparin raw material (9 metric tons/year)
57% China, 20% EU, 14% US, 7% Brazil & Canada, 2% others

Linhardt 2008