



October 21st, 2004

Sacramento, CA

Storm-Klear™ (Chitosan) Toxicity and Applications

Construction Stormwater Treatment

Natural Site Solutions, LLC

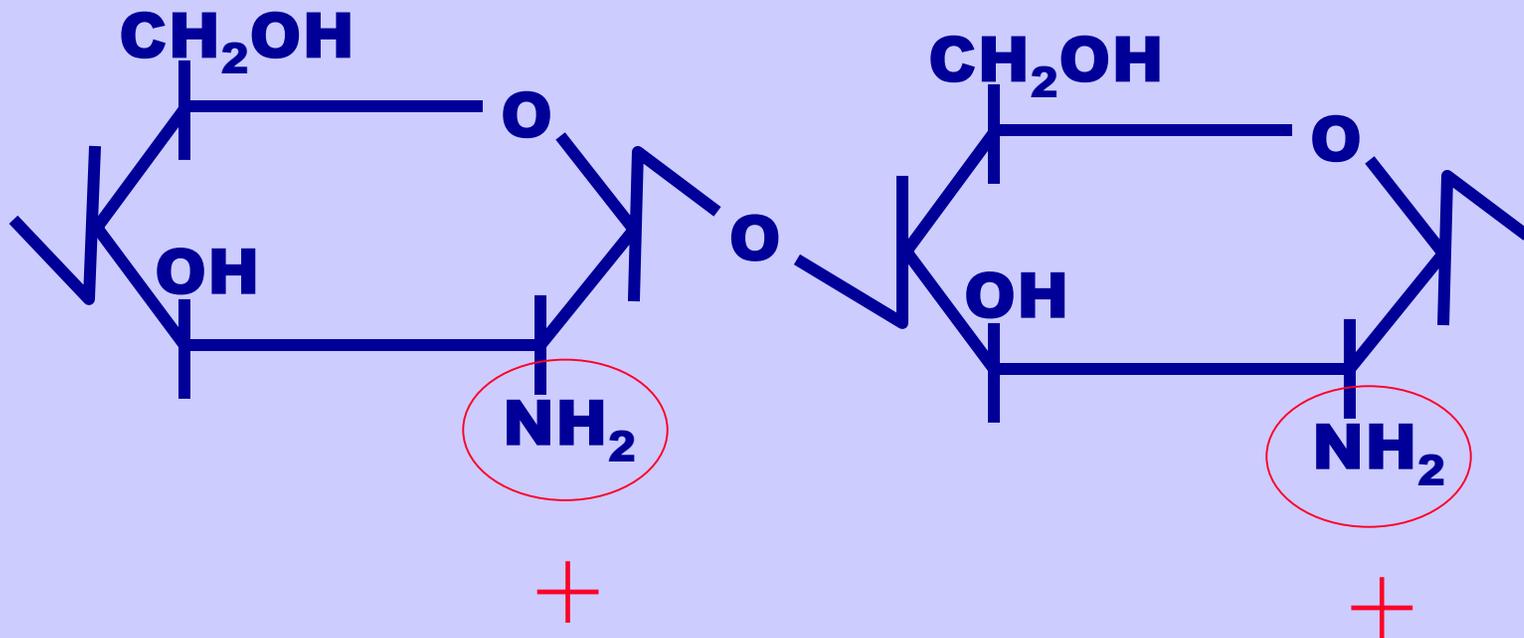
John Macpherson

Presentation Overview:

- Chitosan is a powerful cationic biopolymer.
- Chitosan is removed in the treatment process.
- Residual chitosan is less than 1/10th the toxic threshold.
- Residual chitosan can be detected down to 100 µg/L in treated water using a simple field test.
- Chitosan treatment systems are extremely effective in removing turbidity and other pollutants.

Polyglucosamine (Chitosan) Structure

A cationic polysaccharide biopolymer



Extraction Process

Shell

1



Ground
Shell

2



Chitin

acidic treatment to free calcium

3



alkali treatment to remove proteins

Chitosan

Deacetylation

4



Solubilize w/acetic acid

Commercial Forms of Chitosan

(Chitosan Acetate/Lactate)

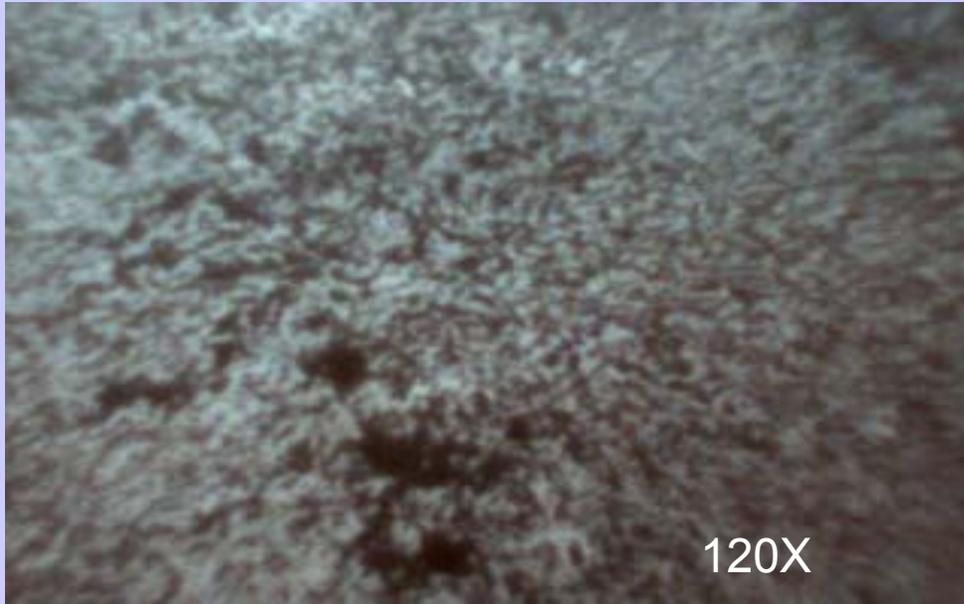
- **Storm-Klear Liqui-Floc™** (1% liquid form)
- **Liqui-Floc™ Concentrate** (2% liquid form)
- **Storm-Klear Gel-Floc™** (100% solid form)

- **Manufactured by Vanson, Inc. of Redmond, Washington**

Chitosan Can Remove:

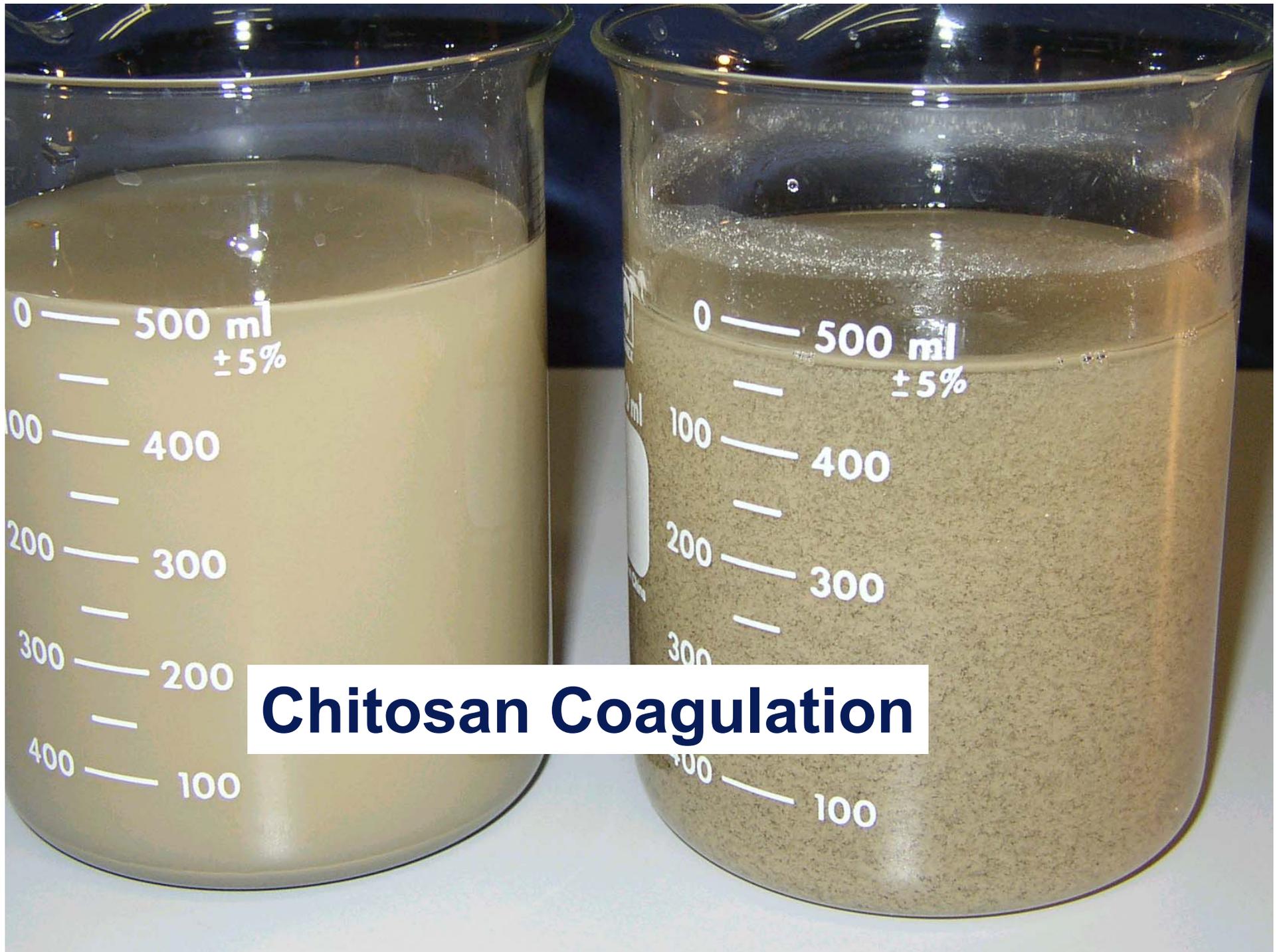
- **Suspended Sediment (turbidity)**
- **Total Phosphorus**
- **Total & Dissolved Metals**
- **PAHs/PCBs/other Organics**
- **Chitosan is effective on 95% of soils tested**

Coagulation - Flocculation



Stormwater contaminated with sediment before and after coagulation with chitosan





Chitosan Coagulation

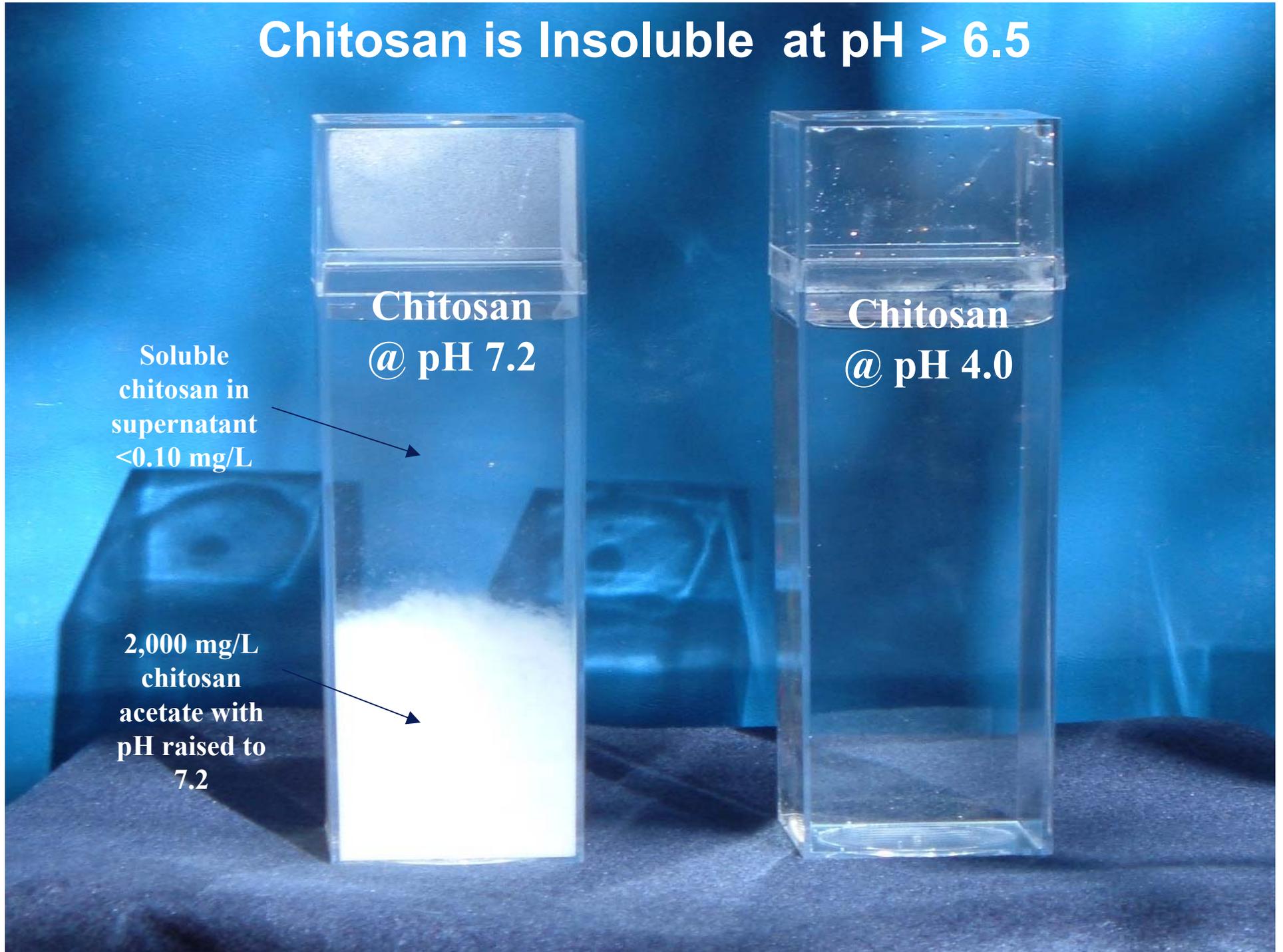
Chitosan is Insoluble at pH > 6.5

Soluble
chitosan in
supernatant
<0.10 mg/L

2,000 mg/L
chitosan
acetate with
pH raised to
7.2

Chitosan
@ pH 7.2

Chitosan
@ pH 4.0





**Cationic starch is
partially soluble
at pH 7.5**

**Anionic PAM is
fully soluble at
pH 7.5**

General Environmental Safety

U.S. EPA Says:

“Given its low potential for toxicity and its abundance in the natural environment, chitosan is not expected to harm people, pets, wildlife, or the environment when used according to label directions.”

...and

“Risks to the environment are not expected because chitosan has not shown toxicity in mammals, it is abundant in nature, and it is used in tiny amounts.”

Source:

http://www.epa.gov/pesticides/biopesticides/ingredientsfactsheets/factsheet_128930.htm Posted June 2003

Chitosan Aquatic Toxicity

Chitosan Acetate

Typical dose rate 0.3 to 3.0 mg/L



**Liqui-Floc (chitosan acetate) Definitive Aquatic Toxicity Results
(in clean water)**

TEST ORGANISM	TEST ENDPOINT	EC50 OR IC50	EC25 ¹ OR IC25 ¹
Fathead minnow	96-hr survival	6.42 mg/L	NC ²
	7-day survival	>10.0 mg/L	NC
	7-day growth	>10.0 mg/L	NC
	7-day teratogenicity	10 – 100 mg/L	NC
Daphnia pulex	48-hr survival	13.69 mg/L	NC
Rainbow trout	96-hr survival	1.73 mg/L	1.28 mg/L
	* 7-day survival	1.54 mg/L	1.21 mg/L
	7-day growth	1.52 mg/L	1.16 mg/L
	7-day embryo	>10.0 mg/L	NC

¹ Toxic Threshold.

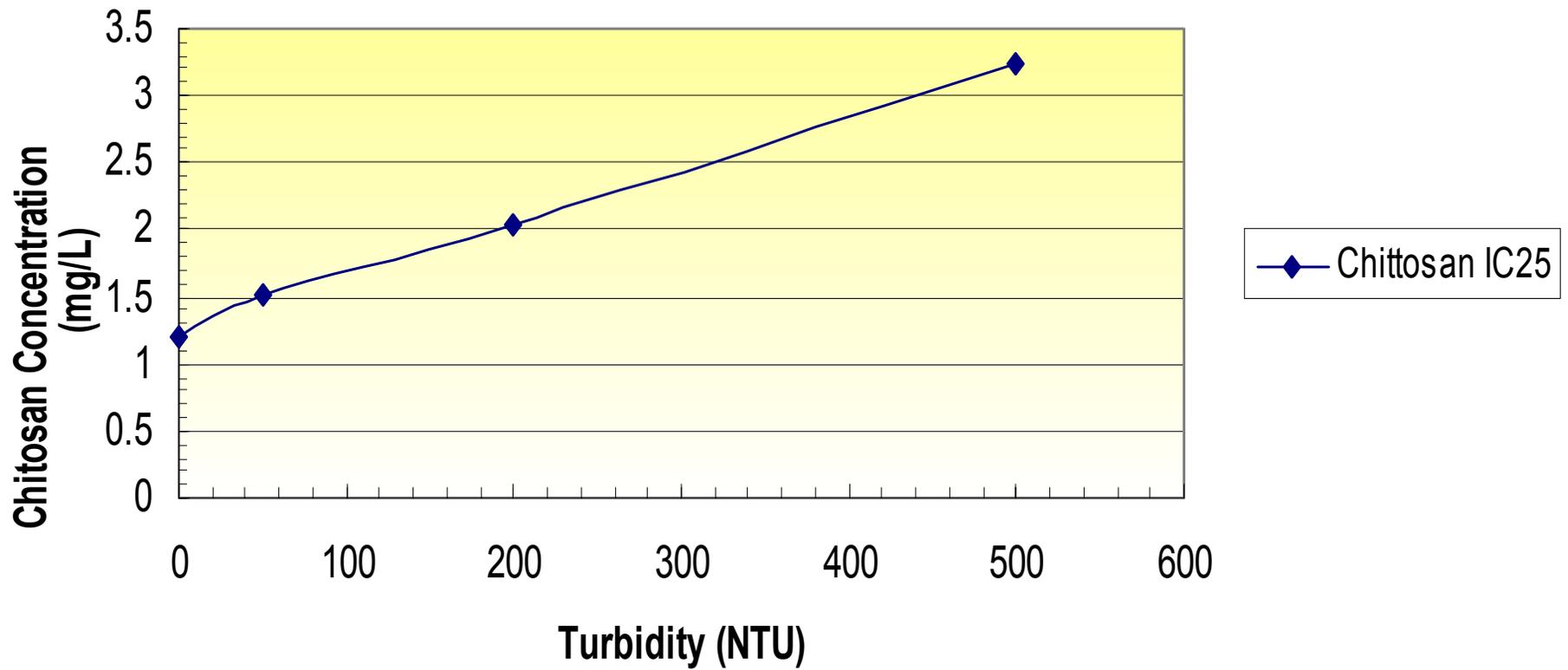
² NC = not calculated because they do not represent the most sensitive species.

Liqui-Floc (mg/L)	Control	50 NTU	200 NTU	500 NTU
0	100.0 ± 0.0	97.5 ± 5.0	100.0 ± 0.0	100.0 ± 0.0
0.25	100.0 ± 0.0	--	--	--
0.50	100.0 ± 0.0	100.0 ± 0.0	--	--
1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
2.0	25.0 ± 10.0	57.5 ± 15.0	100.0 ± 0.0	100.0 ± 0.0
4.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	67.5 ± 22.2
8.0	--	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
16.0	--	--	0.0 ± 0.0	0.0 ± 0.0
Chronic (7-day) Rainbow trout survival test in turbid water.				
LC50	1.68	2.11	2.83	4.52
Toxic threshold*	1.21	1.52	2.03	3.25

* Defined as lowest 25% point estimate – in these tests it is equivalent to the LC25

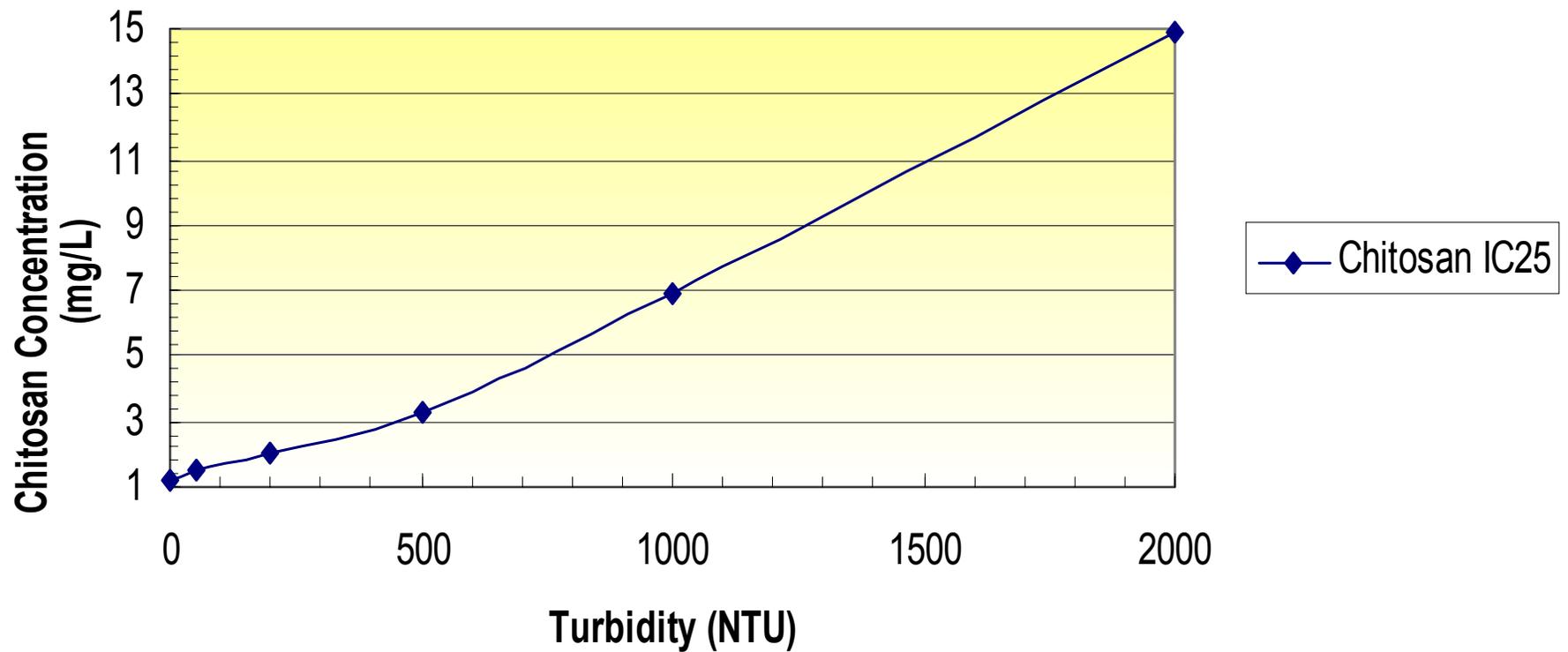
Chitosan Acetate IC25 -v- Turbidity

(Chronic – Rainbow trout)



Chitosan Acetate IC25 -v- Turbidity Extrapolated

(Chronic – Rainbow trout)



Exaggerated Chitosan Dose Toxicity Test

TEST # 03-19-99 (20.4 mg/L dose rate)							
Time	Dose Rate ($\mu\text{g/L}$)	Before Filtration			After Filtration		
		Turbidity (NTU)	pH	Conductivity ($\mu\text{S/cm}$)	Turbidity (NTU)	pH	Conductivity ($\mu\text{S/cm}$)
1440	20,400	643	7.1	143	3.1	7.1	111
Whole effluent exhibited no acute mortality to Rainbow trout or <i>Daphnia magna</i> .							

What does all this Mean?

For the most sensitive species:

The residual chitosan concen. < 0.10 mg/L

The toxic threshold is 1.21 mg/L

The chitosan safety factor is > 12.1

EPA considers a safety factor > 3 adequate

Liqui-Floc (chitosan acetate)

Chitosan Aquatic Toxicity

Chitosan Lactate (Gel-Floc)

Typical dose rate 0.3 to 3.0 mg/L



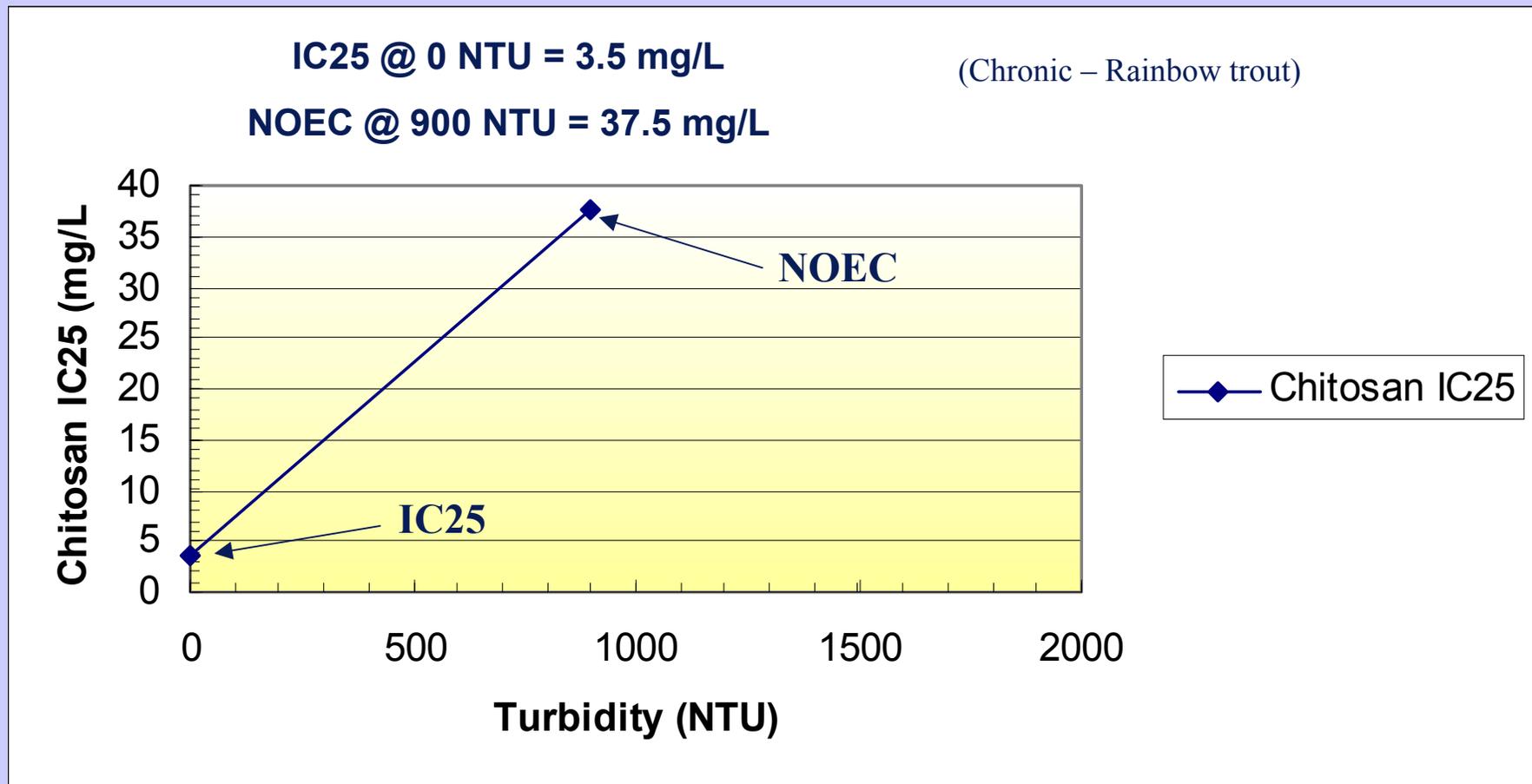
**Gel-Floc (chitosan lactate) Definitive Aquatic Toxicity Results
(in clean water)**

TEST ORGANISM	TEST ENDPOINT	EC50 OR IC50	EC25 ¹ OR IC25 ¹
Fathead minnow	96-hr survival	23 mg/L	NC ²
	7-day survival	25 mg/L	NC
	7-day growth	23 mg/L	NC
	7-day teratogenicity	10 – 100 mg/L	NC
Daphnia pulex	48-hr survival	93 mg/L	NC
Rainbow trout	96-hr survival	6.4 mg/L	4.4 mg/L
	* 7-day survival	5.3 mg/L	4.8 mg/L
	7-day growth	4.0 mg/L	3.5 mg/L
	7-day embryo	>1,000 mg/L	NC

¹ Toxic Threshold.

² NC = not calculated because they do not represent the most sensitive species.

Gel-Floc (chitosan lactate) Dose, Toxicity, Turbidity



And Again, What does all this Mean?

For the most sensitive species:

The residual chitosan concen. <0.10 mg/L

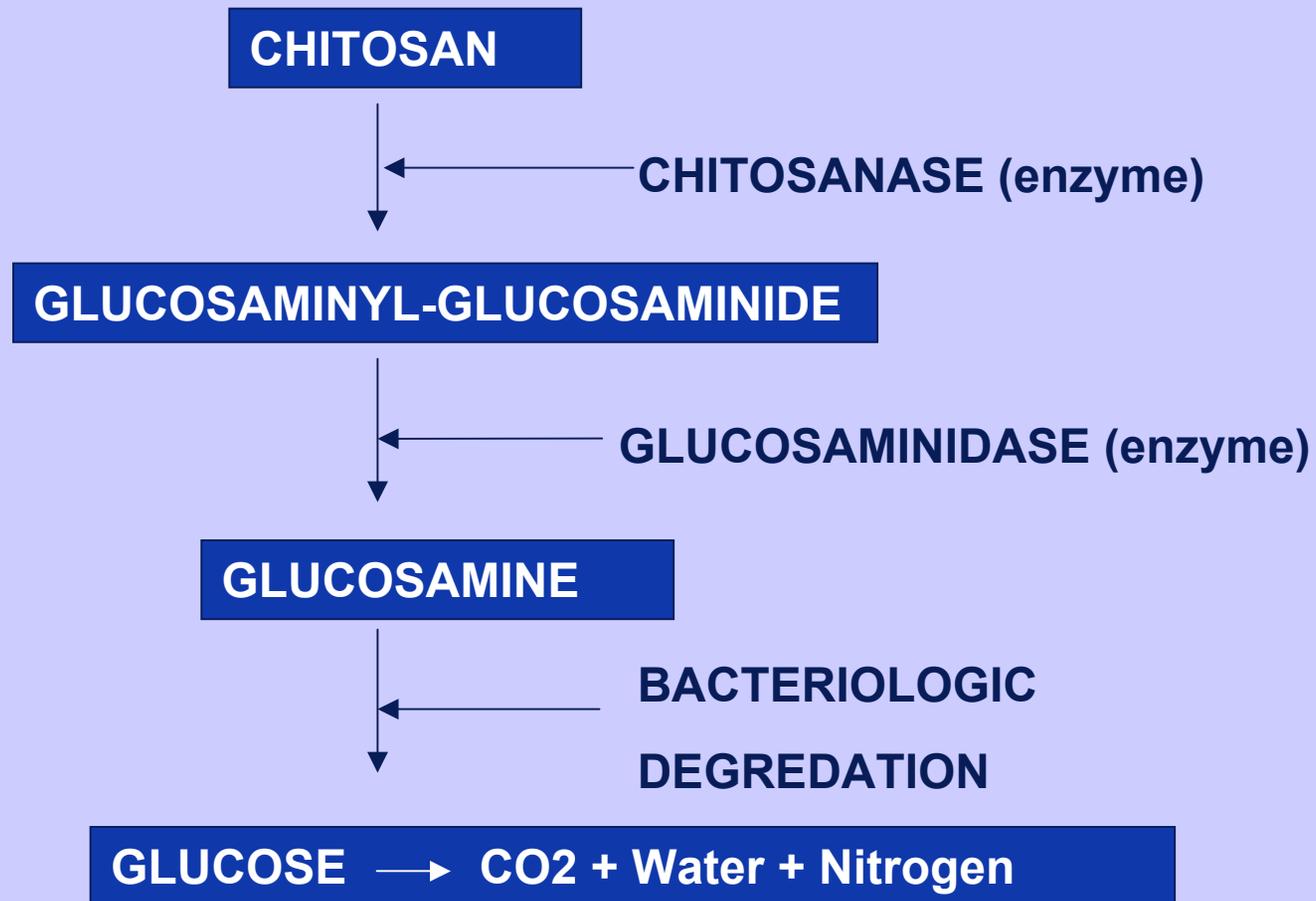
The toxic threshold is 4.8 mg/L

The chitosan safety factor is 48

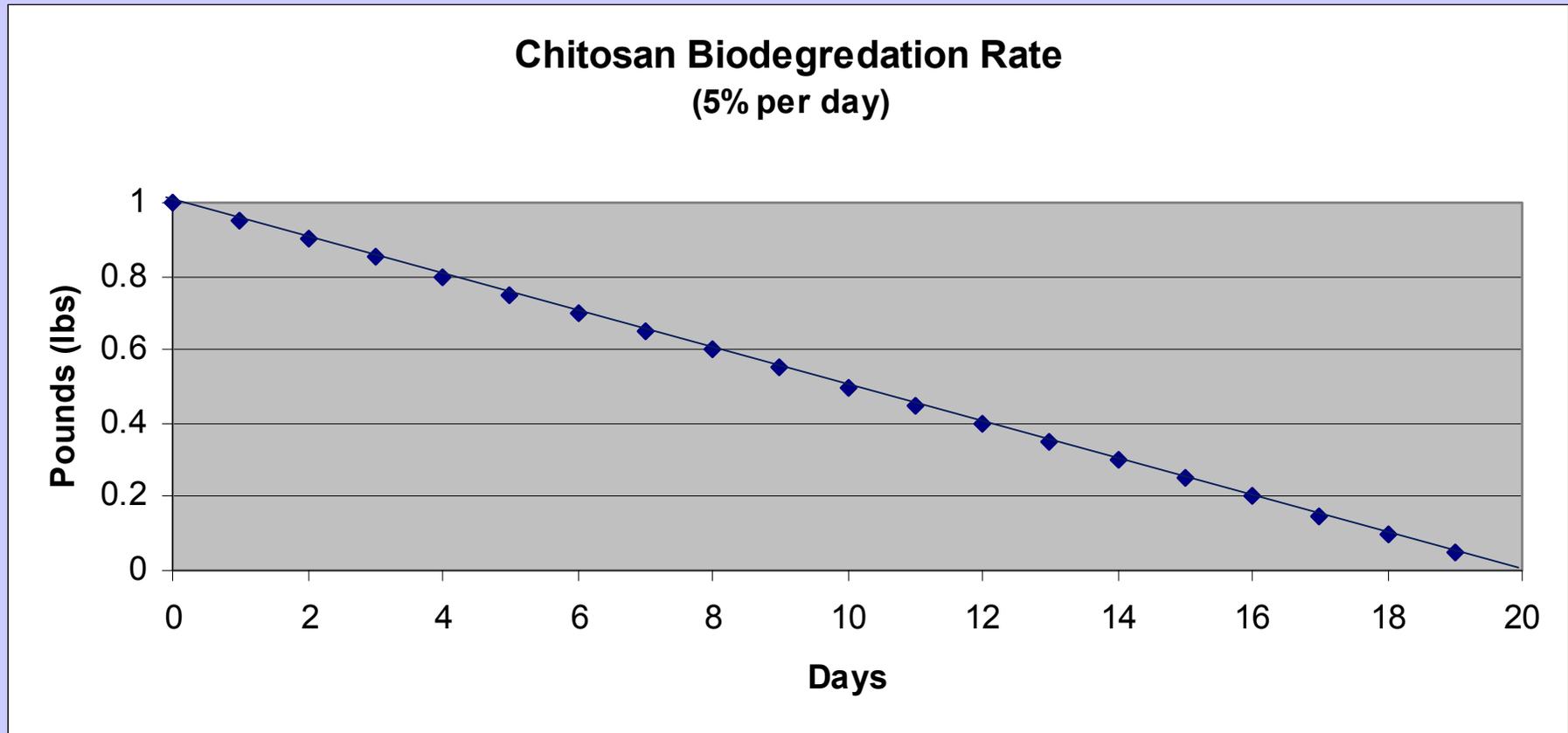
EPA considers a safety factor > 3 adequate

Gel-Floc (chitosan lactate)

BIOLOGICAL DEGRADATION OF CHITOSAN

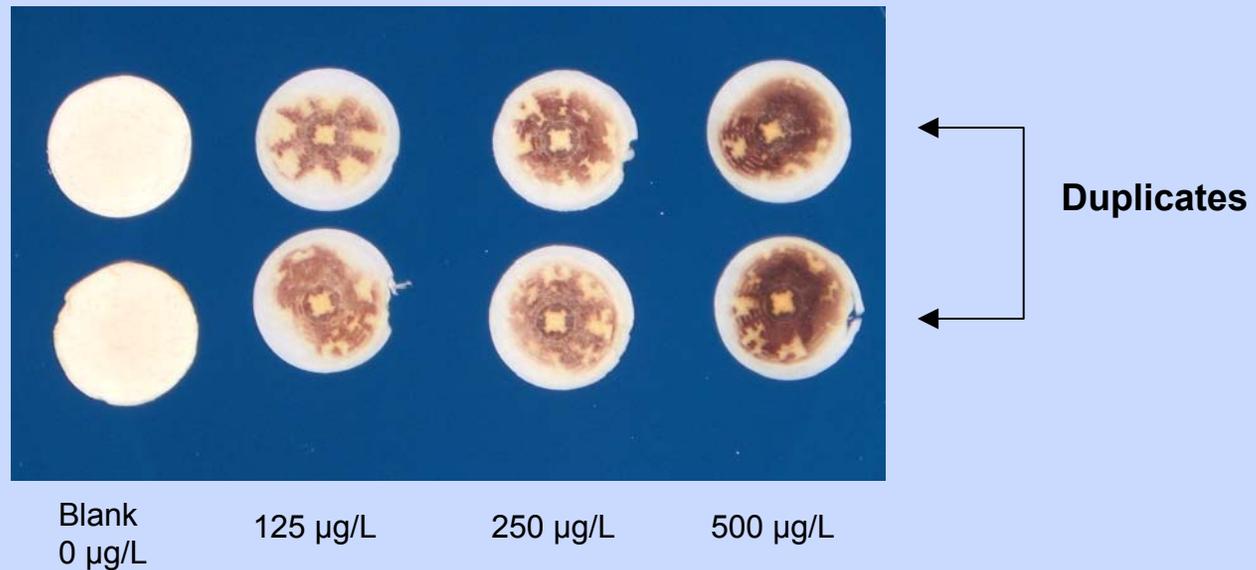


Chitosan Biodegradation (1% to 10% per Day)



Residual Chitosan Test

Based on the reaction between iodine and polysaccharides



Over 1,000 analyses performed with no positive reactions

Engineered Applications



Two Forms of Chitosan Available



Gel-Floc

Passive
Dissolving
Chitosan Gel

Liqui-Floc

Liquid Chitosan
for Injection



Gel-Floc Sock
Installed



Chitosan-Enhanced Sand Filtration



Modular Sand Filter System



Clearwater Compliance
Services, Inc.



CUD Test Project

Lakeside Divisions 1, 2 & 3

Redmond, Washington

Lakeside Test Project Stats

Average turbidity reduction: 98.4% (SD=1.29%, CV=0.013)

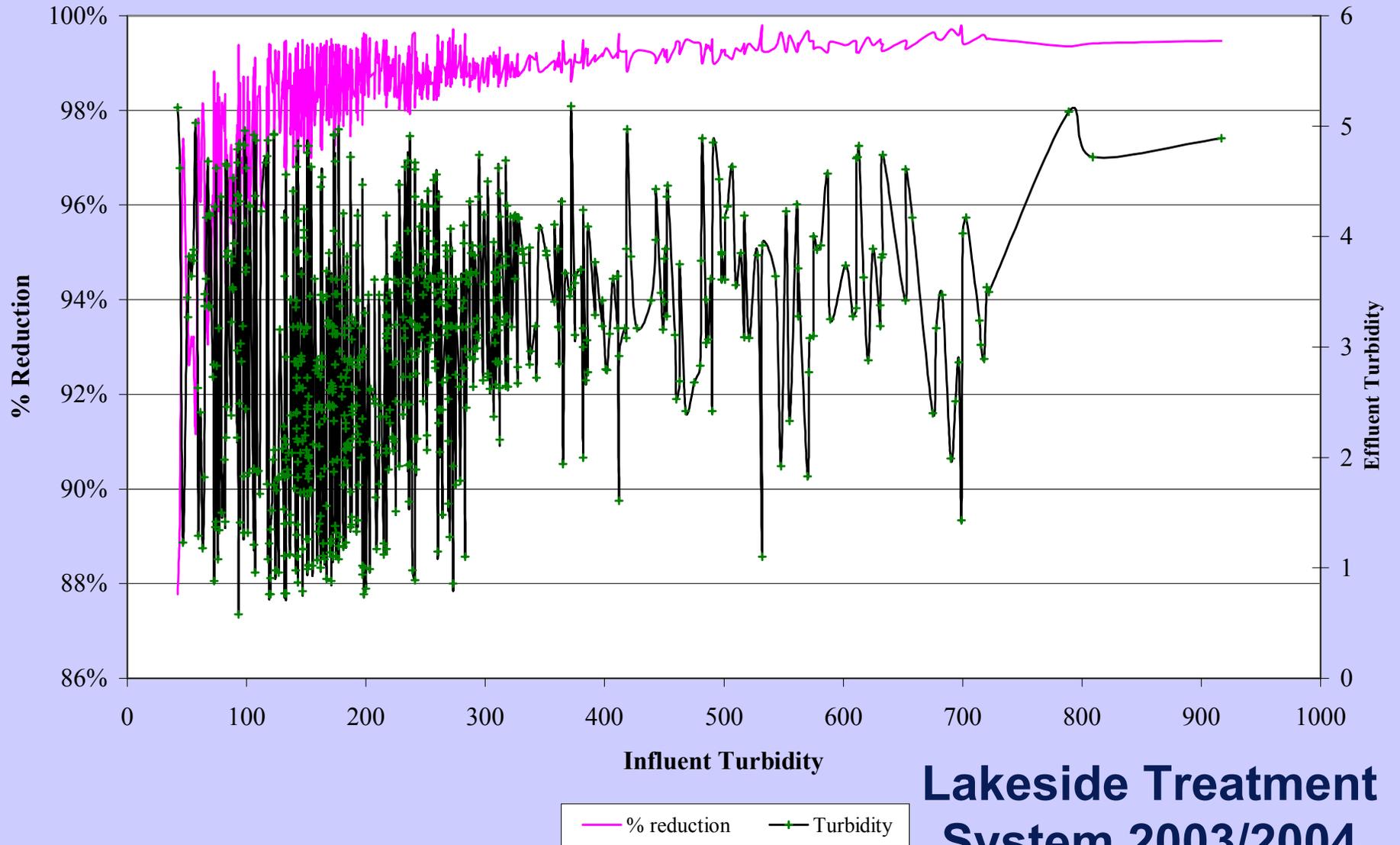
Average influent turbidity: 248 NTU (42.3 NTU to 917 NTU)

Average effluent turbidity: 2.98 NTU (0.58 NTU to 5.18 NTU)

Residual chitosan concn.: <0.10 mg/L

Chitosan dose rate variation: 0.3 mg/L to 0.8 mg/L relative to influent turbidity

Influent Turbidity Vs. % reduction



Lakeside Treatment System 2003/2004

Lakeside Treatment System Acute Trout Results

Rainbow Trout 96-Hour Acute Toxicity

Sample Date	Sample ID	pH	Cond	Turb	Temp	DO	% Survival
2/5/03	LSB-2/5/03-5-E	7.78	145.1	1.93	4.2	9.95	100
2/18/03	LSB-2/18/03-11-E	7.75	173.1	1.1	9	12.59	100
2/21/03	LSB-2/21/03-17-E	7.76	181.1	1.79	9.2	10.63	100
2/21/03	LSB-2/21/03-23-E	7.75	180.4	0.84	9.1	10.98	100
2/28/03	LSB-2/28/03-21-E	7.64	176.6	0.6	8	12.27	100
3/5/03	LSB-3/5/03-52-E	7.73	175.7	2.31	8.7	10.98	100
3/11/03	LSB-3/11/03-85-E	7.71	172.2	4.08	10.3	10.63	100
3/12/03	LSB-3/12/03-115-E	7.63	161.7	1.79	12.6	12.21	100
3/13/03	LSB-3/13/03-145-E	7.61	104.8	1.52	11.9	11.81	100

Lakeside Treatment System Acute Daphnia Results

Daphnia Magna 48-Hour Acute Toxicity

Sample Date	Sample ID	pH	Cond	Turb	Temp	DO	% Survival
2/5/03	LSB-2/5/03-5-E	7.78	145.1	1.93	4.2	9.95	97.5
2/18/03	LSB-2/18/03-11-E	7.75	173.1	1.1	9	12.59	100
2/21/03	LSB-2/21/03-17-E	7.76	181.1	1.79	9.2	10.63	95
2/21/03	LSB-2/21/03-23-E	7.75	180.4	0.84	9.1	10.98	100
2/28/03	LSB-2/28/03-21-E	7.64	176.6	0.6	8	12.27	97.5
3/5/03	LSB-3/5/03-52-E	7.73	175.7	2.31	8.7	10.98	97.5
3/11/03	LSB-3/11/03-85-E	7.71	172.2	4.08	10.3	10.63	95
3/12/03	LSB-3/12/03-115-E	7.63	161.7	1.79	12.6	12.21	100
3/13/03	LSB-3/13/03-145-E	7.61	104.8	1.52	11.9	11.81	100
3/24/03	LSB-3/24/03-205-E	7.56	152.6	2.04	9.8	10.91	90

Phosphorus Results

14 Phosphorus tests:

Average % P reduction = 70.4%

Min. = 23.8% reduction

Max. = 93.3 reduction

Median = 78.6% reduction

Seatac Int'l Airport Seattle

3,500 gpm Storm-Klear™ Sand Filtration System



One Of Seven (600 gpm) Chitosan Treatment Systems Installed at Seatac Int'l Airport



Conclusions

- Chitosan is an effective coagulation/flocculation agent.
- Chitosan dose rates are 0.3 to 3 mg/L.
- **Chitosan is removed with sediment in the treatment process.**
- Chitosan has a conservative safety factor > 10 .
- **Water treated with chitosan has never exhibited toxicity.**
- Chitosan treatment systems are extremely effective in the purification of construction stormwater.