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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

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OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

**MEMORANDUM**

**SUBJECT:** Review of Ecotoxicology Studies to Support Registration of **Silver Sodium Hydrogen Zirconium Phosphate (AlphaSan RC 5000)**

**TO:** Dennis Edwards, Chief  
Marshall Swindell, Product Manager / PM Team 33  
Regulatory Management Branch I  
Antimicrobials Division (7510 C)

**FROM:** Wanda Jakob, Biologist *Wanda Jakob 02/13/01*  
Team Two  
Risk Assessment and Science Support Branch (RASSB)  
Antimicrobials Division (7510 C)

**THRU:** Najm Shamim, Acting Team Leader *A Shamim 2/14/2001*  
Team Two  
Risk Assessment and Science Support Branch (RASSB)  
Antimicrobials Division (7510 C)

Norm Cook, Chief *Norm Cook 02/15/01*  
Risk Assessment and Science Support Branch (RASSB)  
Antimicrobials Division (7510 C)

**Registrant:** Milliken Chemical

**DP Barcode:** D270358

**Pesticide  
Chemical No.:** 072560

**A.I. Chemical  
Name:** Silver Sodium Hydrogen Zirconium Phosphate

**EPA Reg. No.:** 011631-00002

1/27

**PURPOSE OF ACTION:** Review ecotoxicology data evaluation reports (DER's) to support registration of silver sodium hydrogen zirconium phosphate (AlphaSan RC 5000).

**BACKGROUND:** Milliken Chemical has submitted two ecotoxicity studies to the Agency for review and evaluation of toxicity to rainbow trout and *Daphnia magna*. The studies include:

1. 72-1 - Acute Coldwater Fish LC<sub>50</sub> Test (*Onchorynchus mykiss*): MRID 452523-01
3. 72-2 - Acute Toxicity Test: Freshwater Invertebrate (*Daphnia magna*); MRID 452523-02

**SUMMARY / ATTACHED DATA EVALUATION REPORTS (DER's):**

1. The 96 hour acute freshwater fish (rainbow trout) study indicates silver sodium hydrogen zirconium phosphate (AlphaSan RC 5000) is **highly toxic** to rainbow trout with an LC<sub>50</sub> of 0.643 ppm. This study is scientifically sound and fulfills guideline requirements for a freshwater fish LC<sub>50</sub> test and is classified as **Core**.

**Recommendation:** None

2. The acute toxicity for freshwater invertebrate (*Daphnia magna*) study indicates silver sodium hydrogen zirconium phosphate (AlphaSan RC 5000) is **very highly toxic** to *Daphnia magna* with an EC<sub>50</sub> of 0.0 23 ppm. This study is scientifically sound and fulfills guideline requirements for a freshwater invertebrate EC<sub>50</sub> test and is classified as **Core**.

**Recommendation:** None

3. **NOTE:** Environmental Hazard Statements are required by the Agency [40 CFR Part 156.10 (2)(B)(ii)] where an "environmental hazard exists to non-target organisms, precautionary statements are required stating the nature of the hazard and the appropriate precautions to avoid potential accidents, injury or damage." RASSB defers to the Office of Water regarding appropriate environmental hazards labeling as it pertains to possible discharge of effluent into lakes, rivers, streams, ponds, estuaries, marshes, oceans, or to other waters or into sewer systems.

**Recommendation:** The environmental hazard statement/paragraph remain on the label.

DP Barcode: D270358

MRID No.: 452523-01

**DATA EVALUATION RECORD**  
**OPP § 72-1(c), OPPTS 850.1075**  
**ACUTE LC<sub>50</sub> TEST WITH A COLDWATER FISH**

1. **CHEMICAL:** Silver Sodium Hydrogen  
Zirconium Phosphate (072560)

PC Code No.: 072560

2. **TEST MATERIAL:** AlphaSan™ RC 2000, Lot 7180215

Purity: 33.1% zirconium  
9.8% silver; by weight

3. **CITATION**

Authors: Susan J. Palmer, Raymond L Van Hoven, Ph.D., Henry O. Krueger, Ph.D.

Title: Alphasan RC 2000: A 96-Hour Static Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*)

Study Completion Date: October 27, 2000

Laboratory: Wildlife International, Ltd., 8598 Commerce Dr., Easton, MD 21601

Sponsor: Milliken Chemical, 920 Milliken Rd., P.O. Box 1927, Spartanburg, SC 29304-1927

Laboratory Project ID: 522A-102A

MRID No.: 452523-01

DP Barcode: D270358

4. **REVIEWED BY:** W. Jakob, Biologist, Antimicrobials Division (7510C)

Signature: *Wanda Jakob*

Date: 02/13/01

5. **APPROVED BY:** Norm Cook, Chief, Antimicrobials Division (7510C)

Signature: *Norm Cook*

Date: 02/15/01

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:

*Oncorhynchus mykiss*

Age or Size of Test Organism:

Mean weight (of control): 1.12 g  
(blotted dry);

mean length (of control) 4.4 cm

Definitive Test Duration:

96 hours (10/9/2000 to 10/13/2000)

Study Method:

Static

Type of Concentrations:

Nominal and measured

DP Barcode: D270358

MRID No.: 452523-01

## 7. CONCLUSIONS

The acute toxicity for freshwater fish (*Oncorhynchus mykiss*) study indicates silver sodium hydrogen zirconium phosphate is **highly toxic** to rainbow trout with an LC<sub>50</sub> of 0.643 ppm and an NOEC of 0.236 ppm. This study is scientifically sound and fulfills guideline requirements for a freshwater fish LC<sub>50</sub> test and is classified as **Core**.

### Results Synopsis

24-hr LC<sub>50</sub>: 1292 µg/L AlphaSan  
45-hr LC<sub>50</sub>: 661 µg/L  
72-hr LC<sub>50</sub>: 661 µg/L  
96-hr LC<sub>50</sub>: 643 µg/L  
NOEC: 236 µg/L

95% C.I.: 948 - 2290 µg/L AlphaSan  
95% C.I.: 501 - 948 µg/L  
95% C.I.: 501 - 948 µg/L  
95% C.I.: 501 - 948 µg/L  
Probit Slope: Not appropriate

### Verified Results Synopsis

24-hr LC<sub>50</sub>: 1485 µg/L AlphaSan  
45-hr LC<sub>50</sub>: 707 µg/L  
72-hr LC<sub>50</sub>: 707 µg/L  
96-hr LC<sub>50</sub>: 689 µg/L  
NOEC: 236 µg/L

95% C.I.: 1345 - 1625 µg/L AlphaSan  
95% C.I.: 673 - 741 µg/L  
95% C.I.: 673 - 741 µg/L  
95% C.I.: 642 - 735 µg/L  
Probit Slope: Not appropriate

## 8. ADEQUACY OF THE STUDY

- A. **Classification:** Core
- B. **Rationale:** NA.
- C. **Repairability:** NA.

## 9. GUIDELINE DEVIATIONS

1. Three fish placed in the two highest concentration chambers were accidentally fed within the 24-hour period prior to the test. All other fish were not fed during the acclimation period (at least 48 hours) or during the test. We do not consider this to be a major guideline deviation.

## 10. SUBMISSION PURPOSE: Registration

## 11. MATERIALS AND METHODS

### A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> Preferred species is the rainbow trout ( <i>Oncorhynchus mykiss</i> )	Rainbow trout ( <i>Oncorhynchus mykiss</i> )
<u>Mean Weight</u> 0.5 - 5 g	Average (of control): 1.12 g Range (of control): 0.65 - 1.78 g

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Guideline Criteria	Reported Information
<u>Mean Standard Length</u> Longest not > 2x shortest	Average (of control): 4.4 cm Range (of control): 3.8 - 5.0 cm Longest was not > 2x shortest
Supplier	Thomas Fish Company, Anderson, California
All fish from same source?	Yes
All fish from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
<u>Acclimation Period</u> Minimum 14 days	Acclimated to test conditions for 53 hours; held for approximately 14 weeks prior to test under similar temperature and pH conditions
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	No
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
<u>Feeding</u> No feeding during the study	No feeding during the study
<u>Pretest Mortality</u> < 3% mortality 48 hr prior to testing	0%

C. Test System

Guideline Criteria	Reported Information
<u>Source of dilution water</u> Soft reconstituted water or water from a natural source, <i>not</i> dechlorinated tap water	Well water, passed through a sand filter (pore size 25 $\mu\text{m}$ ) and aerated in storage tank. Prior to use, filtered to 0.45 $\mu\text{m}$ to remove microorganisms. Water then diluted with reverse osmosis-treated water to reduce hardness to between 50 and 60 mg/L as $\text{CaCO}_3$ .
Does water support test animals without observable signs of stress?	Not reported (during the definitive test, no mortality and no abnormal behavior was observed in the controls).

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Guideline Criteria	Reported Information
<u>Water Temperature</u> 12°C	11 - 13.8°C
<u>pH</u> Prefer 7.2 to 7.6	7.8-8.2
<u>Dissolved Oxygen</u> Static: ≥ 60% during 1 <sup>st</sup> 48 hr and ≥ 40% during 2 <sup>nd</sup> 48 hr, flow-through: ≥ 60%	DO ranged from 9.4 - 10.6 mg/L (equivalent to approximately 87 - 103% at 15°C at sea level). Test solutions were aerated.
<u>Total Hardness</u> Prefer 40 to 48 mg/L as CaCO <sub>3</sub>	56 mg/L as CaCO <sub>3</sub> in treatment groups; 60 mg/L as CaCO <sub>3</sub> in control. (Note: OPPTS Guideline 850.1075 specifies 40 to 180 mg/L as CaCO <sub>3</sub> .)
<u>Test Aquaria</u> 1. Material: glass or stainless steel 2. Size: volume of 18.9 L (5 gal) or 30 x 60 x 30 cm 3. Fill volume: 15-30 L of solution	1. Glass 2. 52 L glass aquaria 3. Fill volume: 40 L
<u>Type of Dilution System</u> Must provide reproducible supply of toxicant	Static Test: Aliquots of test substance were weighed and added to the vortex in the dilution water created in each test chamber with a top-down electric mixer.
<u>Flow Rate</u> Consistent flow rate of 5-10 vol/24 hr, meter systems calibrated before study and checked twice daily during test period	N/A
<u>Biomass Loading Rate</u> Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day	Not reported; reviewer estimated the biomass loading rate to be 0.56 g/L at ≤ 17°C
<u>Photoperiod</u> 16 hours light, 8 hours dark	16 hours light, 8 hours dark
<u>Solvents</u> Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	None

D. Test Design

Guideline Criteria	Reported Information
<u>Range Finding Test</u> If LC <sub>50</sub> > 100 mg/L with 30 fish, then no	Range finding test performed.

Guideline Criteria	Reported Information
<p><b><u>Nominal Concentrations of Definitive Test</u></b> Control &amp; 5 treatment levels; each dosage should be 60% of the next highest concentration; concentrations should be in a geometric series</p>	<p>Control and 6 nominal treatment levels: 78, 160, 310, 630, 1300, and 2500 mg/L. Each dosage is 48-52% of the next highest concentration. Concentrations are in a geometric series.</p>
<p><b><u>Number of Test Organisms</u></b> Minimum 10/level, may be divided among containers</p>	<p>20 fish per test concentration and control</p>
<p>Test organisms randomly or impartially assigned to test vessels?</p>	<p>Randomly assigned</p>
<p>Biological observations made every 24 hours?</p>	<p>Yes. Observations made approximately 3.5, 24, 48, 72, and 96 hours after test initiation</p>
<p><b><u>Water Parameter Measurements</u></b> 1. Temperature: Measured constantly or, if water baths are used, every 6 hrs, may not vary &gt; 1°C 2. DO and pH: Measured at beginning of test and ever 48 hr in the high, medium, and low doses and in the control</p>	<p>1. Temperature measured continuously in the control test chamber. Temperature was measured in all chambers at the beginning and end of the test, and every 24 hours during the test. 2. DO and pH were measured in all chambers at the beginning and end of the test and at approximate 24 hour intervals during the test.</p>
<p><b><u>Chemical Analysis</u></b> Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used</p>	<p>Yes. AlphaSan was not soluble, although its active ingredient, silver, was soluble. To keep AlphaSan in suspension, test chambers were continuously mixed with magnetic stir bars and plates throughout the experiment. The negative control, 78, 160, and 310 µg/L test solutions were clear and colorless when checked at 0, 24, and 48 hrs. The 630, 1300, and 2500 µ/L test suspensions were slightly cloudy in appearance throughout the test, with cloudiness increasing with increasing concentration. See Reviewer's Comments (Section 14) for description of analytic results.</p>

**Comments:** Total silver and zirconium determinations were made using inductively coupled plasma atomic emission spectrometry (ICP-AES). First, samples of the test solutions were dried and digested with concentrated hydrofluoric and 50% nitric acid. These were brought to volume with dilution solvent and placed in calibrated 50 mL centrifuge tubes and sent for ICP-AES. Emission was monitored at wavelengths of 328.068 and 338.289 nm.



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Dissolved silver and zirconium determinations were made using ICP-AES configured for ultrasonic nebulization sample introduction. First, samples of the test solutions were filtered through a 0.2 $\mu$ m x 13 mm pore filter into the centrifuge tubes. Emission was monitored at wavelengths of 339.197 and 343.823 nm.

Calibration standards were prepared for both silver and zirconium.

**12. REPORTED RESULTS**

**A. General Results**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Recovery of Chemical	Declined over time; see Reviewer's Comments in Section 14.
<u>Control Mortality</u> Not more than 10% control organisms may die or show abnormal behavior.	0%
Raw data included?	No
Signs of toxicity (if any) were described?	Yes (see below)

**Mortality**

Concentration ( $\mu$ g/L)		Number of Fish	Cumulative Number Dead Hour of Study				
Nominal	Measured (24 or 96 hr)		3.5	24	48	72	96
Control	0	20	0	0	0	0	0
Solvent Control	-	-	-	-	-	-	-
78	47	20	0	0	0	0	0
160	126	20	0	0	0	0	0
310	236	20	0	0	0	0	0
630	501	20	0	0	1	1	2
1300	948	20	0	3	20	20	20
2500	2290	20	0	20	20	20	20

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Other observations: One fish in the 948  $\mu\text{g/L}$  mean measured test concentration was reported to be "lying on bottom with little motion other than gill movement" at 24 hours. There were no other visible signs of stress reported.

### B. Statistical Results

Method: The EC<sub>50</sub> and its 95% confidence interval for mortality and immobility (one endpoint) was calculated using the binomial method in the EPA computer program developed by C.E. Stephan. The NOEC was determined by visual inspection of the data.

24-hr LC <sub>50</sub> : 1292 $\mu\text{g/L}$ AlphaSan	95% C.I.: 948 - 2290 $\mu\text{g/L}$ AlphaSan
45-hr LC <sub>50</sub> : 661 $\mu\text{g/L}$	95% C.I.: 501 - 948 $\mu\text{g/L}$
72-hr LC <sub>50</sub> : 661 $\mu\text{g/L}$	95% C.I.: 501 - 948 $\mu\text{g/L}$
96-hr LC <sub>50</sub> : 643 $\mu\text{g/L}$	95% C.I.: 501 - 948 $\mu\text{g/L}$
NOEC: 236 $\mu\text{g/L}$	Probit Slope: Not appropriate

### 13. VERIFICATION OF STATISTICAL RESULTS

Parameter	Results (measured)
Binomial Test LC <sub>50</sub> (C.I.)	Not used
Moving Average Angle LC <sub>50</sub> (95% C.I.)	Not used
Probit LC <sub>50</sub> (95% C.I.)	Data do not support probit analysis
Probit Slope	Data do not support probit analysis
Spearman-Karber 96-hr LC <sub>50</sub> (95% C.I.)	689 (642-735) $\mu\text{g/L}$ AlphaSan
NOEC (mortality)	236 $\mu\text{g/L}$ AlphaSan
NOEC (abnormal behavior)	236 $\mu\text{g/L}$ AlphaSan

24-hr LC <sub>50</sub> : 1485 $\mu\text{g/L}$ AlphaSan	95% C.I.: 1345-1625 $\mu\text{g/L}$ AlphaSan
45-hr LC <sub>50</sub> : 707 $\mu\text{g/L}$	95% C.I.: 673 - 741 $\mu\text{g/L}$
72-hr LC <sub>50</sub> : 707 $\mu\text{g/L}$	95% C.I.: 673 - 741 $\mu\text{g/L}$
96-hr LC <sub>50</sub> : 689 $\mu\text{g/L}$	95% C.I.: 642 - 735 $\mu\text{g/L}$
NOEC: 236 $\mu\text{g/L}$	Probit Slope: Not appropriate

The authors relied on statistical software made available by EPA in 1978. We used the Spearman-Karber method as implemented by TOXSTAT, Version 3.5 (WEST Inc, 1996) to estimate the EC<sub>50</sub>. The Spearman-Karber test is the most appropriate given the data (i.e., one partial mortality and zero mortality in the lowest concentration and 100% mortality at the highest concentration tested). We did not log-transform the concentration data. The result is similar to the value obtained by the authors using the binomial method; however, the 95% C.I. is much smaller.

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**14. REVIEWER'S COMMENTS**

**A. Measured Concentrations: Homogeneity and Stability over Time**

The authors selected silver (Ag) and zirconium (Zr) to represent the test substance due to the insolubility of the polymeric backbone and ion-exchange properties of AlphaSan RC 2000. Results of the analyses for total and dissolved silver are presented in Tables 1 and 2 below. Results of the analyses for total and dissolved zirconium are presented in Tables 3 and 4 below.

Concentrations of total silver and zirconium declined over time. As indicated in Table 1, the concentration of total silver declined from 74.2% - 99.1% of nominal at test initiation, to 51.5% - 83.9% of nominal at 48 hours, reaching 46.0% - 73.5% of nominal at 96 hours. As indicated in Table 3, the concentration of total zirconium declined from 75.2% - 100% at test initiation to 69.3% - 85.1% of nominal at 48 hours, reaching 64.8% - 76.2% of nominal at 96 hours. The authors attributed the decline with time of total silver and zirconium in suspension to the molecules of AlphaSan falling out of suspension. These declines are relatively small, particularly when compared with the declines exhibited in the daphnid toxicity test (MRID No. 452523-02). Thus, the laboratory was reasonably successful at maintaining the AlphaSan in suspension in the fish test aquaria.

Concentrations of dissolved silver exhibited a different pattern. Where quantified, dissolved silver concentrations remained relatively steady over time (Table 2). No dissolved zirconium was detected in any sample. That result was expected because zirconium is part of the polymeric ion exchange backbone of the AlphaSan.

The silver ions in solution represent the active ingredient of AlphaSan.

Table 1. Measured Concentrations of Total Silver<sup>1</sup>

Nominal Concentrations ( $\mu\text{g/L}$ )		Ag Measured ( $\mu\text{g/L}$ )			Measured as % of Nominal		
AlphaSan	Total Ag <sup>2</sup>	0 hr	48 hr	96 hr	0 hr	48 hr	96 hr
78	7.65	6.41	3.94	3.52	83.7	51.5	46.0
160	15.7	14.3	11.3	11.5	91.2	71.9	73.5
310	30.4	27.6	21.1	20.8	90.8	69.3	68.3
630	61.8	56.6	49.9	40.9	91.6	80.7	66.2
1300	128	94.7	91.2	--	74.2	71.5	--
2500	245	243	206	--	99.1	83.9	--

<sup>1</sup>Data from Table 2 of Study Report.<sup>2</sup>Calculated based on 9.81% (w/w) Ag in AlphaSan RC 2000.  
Abbreviations: -- Not measured due to 100% mortality of fish.Table 2. Measured Concentrations of Dissolved Silver<sup>1</sup>

Nominal Concentrations ( $\mu\text{g/L}$ )		Ag Measured ( $\mu\text{g/L}$ )			Measured as % of Nominal Dissolved Ag		
AlphaSan	Total Ag <sup>2</sup>	0 hr	48 hr	96 hr	0 hr	48 hr	96 hr
78	7.65	< LOQ	< LOQ	--	--	--	--
160	15.7	< LOQ	3.86	4.75	--	24.6	30.2
310	30.4	7.50	6.60	7.31	24.7	21.7	24.0
630	61.8	4.00	9.81	12.1	6.47	15.9	19.6
1300	128	21.0	24.1	--	16.5	18.9	--
2500	245	130	78.5	--	53.0	32.0	--

<sup>1</sup>Data from Table 4 of Study Report<sup>2</sup>Calculated based on 9.81% (w/w) Ag in AlphaSan RC 2000.LOQ = limit of quantitation; 0.500 $\mu\text{g}$  Ag/L.

-- Not measured due to 100% mortality of fish.

**Table 3. Measured Concentrations of Total Zirconium <sup>1</sup>**

Nominal Concentrations ( $\mu\text{g/L}$ )		Zr Measured ( $\mu\text{g/L}$ )			Measured as % of Nominal		
AlphaSan	Total Zr <sup>2</sup>	0 hr	48 hr	96 hr	0 hr	48 hr	96 hr
78	25.8	22.8	21.0	16.7	88.5	81.2	64.8
160	53.0	46.6	38.0	40.4	87.9	71.8	76.2
310	103	85.1	71.1	70.3	82.9	69.3	68.5
630	209	178	161	153	85.1	77.1	73.2
1300	430	324	308	–	75.2	71.6	--
2500	828	830	704	–	100	85.1	--

<sup>1</sup>Data from Table 3 of Study Report.<sup>2</sup>Calculated based on 33.1% (w/w) Zr in AlphaSan RC 2000.Abbr: LOQ = limit of quantitation; 2.00  $\mu\text{g Zr/L}$ . – Not measured due to 100% mortality of fish.**Table 4. Measured Concentrations of Dissolved Zirconium <sup>1</sup>**

Nominal Concentrations ( $\mu\text{g/L}$ )		Zr Measured ( $\mu\text{g/L}$ )			Measured as % of Nominal		
AlphaSan	Total Zr <sup>2</sup>	0 hr	48 hr	96 hr	0 hr	48 hr	96 hr
78	25.8	< LOQ	< LOQ	< LOQ	–	–	–
160	53.0	< LOQ	< LOQ	< LOQ	–	–	–
310	103	< LOQ	< LOQ	< LOQ	--	–	–
630	209	< LOQ	< LOQ	< LOQ	–	–	–
1300	430	< LOQ	< LOQ	< LOQ	--	–	–
2500	828	< LOQ	< LOQ	< LOQ	--	–	–

<sup>1</sup>Data from Table 5 of Study Report.<sup>2</sup>Calculated based on 33.1% (w/w) Zr in AlphaSan RC 2000.Abbr: LOQ = limit of quantitation; 2.00  $\mu\text{g Zr/L}$ .

B. Percent Recovery

The authors reported the results of their concurrent quality control measurements based on samples spiked with AlphaSan at 50, 500, and 3000  $\mu\text{g/L}$  and measured for total and dissolved silver and zirconium. For silver, the mean recovery of total silver ranged from 61% to 100% and the mean recovery of the dissolved metal ranged between 72% and 95% (Table 5 below) for the three spiked concentrations tested. For zirconium, the mean recovery of total zirconium from spiked samples ranged from 79.5% to 100% and the mean recovery of dissolved zirconium ranged from 66.0% to 90.4% (Table 6 below). The lowest recovery value of 66% of the dissolved zirconium occurred in the sample spiked at a concentration of 3000  $\mu\text{g/L}$  AlphaSan (estimated 993  $\mu\text{g/L}$  zirconium). The authors expected these results based on the structure of the test substance, which consists of a polymeric ion exchange backbone with relatively low water solubility. Particularly at higher concentrations, molecules of this size were not expected to pass quantitatively through the 0.20  $\mu\text{m}$  pore filter during the analysis of dissolved samples.

The authors did not calculate standard deviations or coefficients of variation (CVs) for the recovery data, so our reviewers did. Table 5 indicates that measurements of total and dissolved silver exhibited coefficients of variation (CVs) between 9.6% and 23.3%. Table 6 indicates that measurements of total and dissolved zirconium were more precise, with CVs of between 3.5% to 12.2% over the same range of AlphaSan concentrations (i.e., 50, 500, and 3000  $\mu\text{g/L}$ ). This range of CVs is acceptable.

Table 5. Recovery of Silver in Quality Control Samples<sup>1</sup>

Nominal Concentration ( $\mu\text{g/L}$ )		Measured Concentration ( $\mu\text{g Ag/L}$ ) <sup>2</sup> (sample number)				Summary Statistics ( $\mu\text{g Ag/L}$ )		
Alpha-San	Ag <sup>3</sup>	(1)	(2)	(3)	(4)	Mean (% Recovery)	Standard Deviation	Coefficient of Variation
Total Silver								
50	4.91	3.72	2.97	2.32	–	3.00 (61.0%)	0.70 (N=3)	23.3
500	49.1	40.7	41.1	34.5	–	38.8 (79.0%)	3.70 (N=3)	9.55
3000	294	289	310	279	–	293 (100%)	15.8 (N=3)	15.8
Dissolved Silver								
50	4.91	3.65	4.06	3.43	3.03	3.54 (72.1%)	0.43 (N=4)	12.1
500	49.1	59.9	45.8	41.1	39.5	46.6 (94.9%)	9.28 (N=4)	19.9
3000	294	282	256	276	193	249 (84.7%)	40.67 (N=4)	16.3

<sup>1</sup>Data from Appendix 3.9 of Study Report; mean, SD, and CV calculated by reviewers.

<sup>2</sup>In each of the samples measured, up to a total of four samples.

<sup>3</sup>Calculated based on 9.81% (w/w) Ag in AlphaSan RC 2000.

Table 6. Recovery of Zirconium in Quality Control Samples<sup>1</sup>

Nominal Concentration ( $\mu\text{g/L}$ )		Measured Concentration ( $\mu\text{g Zr/L}$ ) <sup>2</sup> (sample number)				Summary Statistics ( $\mu\text{g Zr/L}$ )		
Alpha-San	Zr <sup>3</sup>	(1)	(2)	(3)	(4)	Mean (% Recovery)	Standard Deviation (N)	Coefficient of Variation
Total Zirconium								
50	16.6	15.2	13.4	13.6	–	14.1 (84.9%)	0.99 (N=3)	7.01
500	166	138	135	124	–	132 (79.5%)	7.37 (N=3)	5.57
3000	993	983	1030	963	–	992 (100%)	34.4 (N=3)	3.47
Dissolved Zirconium								
50	16.6	15.8	13.2	16.0	–	15.0 (90.4%)	1.56 (N=3)	10.4
500	166	–	–	–	–	NA	NA	NA
3000	993	573	658	733	–	655 (66.0%)	80.1 (N=3)	12.2

<sup>1</sup>Data from Appendix 3.10 of Study Report; mean, SD, and CV calculated by reviewers.

<sup>2</sup>In each of the samples measured, up to a total of four samples.

<sup>3</sup>Calculated based on 33.1% (w/w) Zr in AlphaSan RC 2000.

C. **Conclusions:** The major problems in the companion acute daphnia toxicity test (MRID No. 452523-02) were not problems in this test. The NOEC for active ingredient, dissolved silver ions, was well above the quantitation limit for silver. The loss of AlphaSan from suspension over time was only slight, much less than in the case of the daphnid test. We therefore classify this test as core.

DP BARCODE: D270358

CASE: 062473  
SUBMISSION: S587990

DATA PACKAGE RECORD  
BEAN SHEET

DATE: 02/16/01  
Page 1 of 1

\* \* \* CASE/SUBMISSION INFORMATION \* \* \*

CASE TYPE: REGISTRATION ACTION: 325 AMD-AD ME-TOO USE DAT REQ  
CHEMICALS: 072560 Silver sodium hydrogen zirconium phosphate (Ag0.18 99.0000%

ID#: 011631-00002 AlphaSan RC 5000  
COMPANY: 011631 MILLIKEN CHEMICAL  
PRODUCT MANAGER: 33 MARSHALL SWINDELL 703-308-6341 ROOM: CS1 6B  
PM TEAM REVIEWER: TONY KISH 703-308-9443 ROOM: CS1 3W69  
RECEIVED DATE: 11/01/00 DUE OUT DATE: 02/19/01

\* \* \* DATA PACKAGE INFORMATION \* \* \*

DP BARCODE: 270358 EXPEDITE: N DATE SENT: 11/09/00 DATE RET.: 02/15/01  
CHEMICAL: 072560 Silver sodium hydrogen zirconium phosphate (Ag0.18Na0.57H0.  
DP TYPE: 001

ASSIGNED TO	CSF: Y	LABEL: Y	ADMIN DUE DATE: 01/18/01
DIV : AD	DATE IN	DATE OUT	NEGOT DATE: / /
BRAN: RASSB	11/09/00	02/15/01	PROJ DATE: / /
SECT: RASSB2	11/13/00	02/15/01	
REVR : WJAKOB	11/13/00	02/14/01	
CONTR:	/ /	02/13/01	

\* \* \* DATA REVIEW INSTRUCTIONS \* \* \*

NAJM: We need a review of attached daphnia study (MRID 45252302), and trout study (MRID 45252301) done on related 10% silver containing product. Can reviewer conclude that because this is an indoor use, the existing "Environmental Hazard" statement can be deleted from current label? If so, please have reviewer so state in review. Any questions, see Tony. THANKS!

\* \* \* DATA PACKAGE EVALUATION \* \* \*

Wanda: Please review the packages: MRID#:45252302 and MRID#:45252301.

\* \* \* ADDITIONAL DATA PACKAGES FOR THIS SUBMISSION \* \* \*

DP BC	BRANCH/SECTION	DATE OUT	DUE BACK	INS	CSF	LABEL
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DP Barcode: D270358

MRID No.: 452523-02

**DATA EVALUATION RECORD**  
**OPP § 72-2, OPPTS 850.1010**  
**ACUTE LC<sub>50</sub> TEST WITH A FRESHWATER INVERTEBRATE**

1. **CHEMICAL:** Silver Sodium Hydrogen  
Zirconium Phosphate (072560)

PC Code No.: 072560

2. **TEST MATERIAL:** AlphaSan™ RC 2000, Lot 7180215

Purity: 33.1% zirconium  
9.8% silver; by weight

3. **CITATION**

Authors: Susan J. Palmer, Raymond L. Van Hoven, Ph.D., Henry O. Krueger, Ph.D.

Title: Alphasan RC 2000: A 48-Hour Static Acute Toxicity Test with the Cladoceran (*Daphnia magna*)

Study Completion Date: October 27, 2000

Laboratory: Wildlife International, Ltd., 8598 Commerce Drive, Easton, Maryland 21601

Sponsor: Milliken Chemical, 920 Milliken Rd., P.O. Box 1927, Spartanburg, SC 29304-1927

Laboratory Report ID: 522A-101

MRID No.: 452523-02

DP Barcode: D270358

4. **REVIEWED BY:** Wanda Jakob, Biologist, Antimicrobials Division (7510C)

Signature: *Wanda J. Jakob*

Date: 02/13/01

5. **APPROVED BY:** Norm Cook, Chief, Antimicrobials Division (7510C)

Signature: *Norm Cook*

Date: 02/15/01

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:

*Daphnia magna*

Age of Test Organism:

≤ 24 hr

Definitive Test Duration:

48 hr (10/03/00-10/05/00)

Study Method:

Static

Type of Concentrations:

Nominal and Mean Measured

7. **CONCLUSIONS**

The acute invertebrate toxicity for *Daphnia magna* indicates silver sodium hydrogen zirconium phosphate is **very highly toxic** to daphnids with an EC50 of 0.023 ppm and an NOEC of 0.015 ppm. This study is scientifically sound and fulfills guideline requirements for an acute invertebrate EC<sub>50</sub> test and is classified as **Core**.

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Results Synopsis

24-hr EC<sub>50</sub>: 46 µg/L  
48-hr EC<sub>50</sub>: 23 µg/L  
NOEC: 15 µg/L

95% C.I.: 18 - 93 µg/L  
95% C.I.: 18 - 42 µg/L  
Probit Slope: N/A

Verified Results Synopsis

24-hr EC<sub>50</sub>: 52.5 µg/L  
48-hr EC<sub>50</sub>: 27 µg/L  
NOEC: 15 µg/L

95% C.I.: 44 - 61 µg/L  
95% C.I.: 25 - 30 µg/L  
Probit Slope: N/A

**8. ADEQUACY OF THE STUDY**

A. Classification: Core

B. Rationale: Although the measurements of the active ingredient, dissolved silver ions, in the test solutions were below the detection limit at the LOEC of 18 µg/L AlphaSan, the authors appear to have taken all reasonable approaches to measuring the concentration of the test substance, a polymeric ion-exchange substance, and the active ingredient in the test solutions. Although the AlphaSan appears to have slowly settled out of solution, again, the authors took all reasonable steps to attempt to keep it suspended and to measure its loss from the water column over time. They also used the mean measured values over the duration of the experiment to estimate the EC<sub>50</sub>, as is appropriate. Thus, we conclude that the difficulties identified in this study are inherent in the properties of the test material itself in an aqueous medium, and it might not be possible to replicate these results.

C. Repairability: NA

**9. GUIDELINE DEVIATIONS**

1. The nominal concentrations of each dosage in the definitive test were 50% of the next highest concentration. The guidelines state that each dosage should be 60% of the next highest concentration. We do not consider this to be a major guideline deviation.
2. The temperature was measured continuously during the first 12 hours of the test only in the control chamber due to a malfunction of the temperature recording apparatus (stated in Appendix 4 of the Study Report). This is not a major deviation.
4. The concentration of dissolved silver at the LOEC of 18 µg/L AlphaSan was below the limit of quantitation of for silver.
3. The concentrations of the "test substance" in suspension declined to only 10% to 20% of the nominal concentrations by 48 hours.
3. Although the test substance was not renewed, the test beakers were set up as for a flow-through design. The beakers were open to the full test chamber, where stir bars were used in an attempt to keep the AlphaSan in suspension in the test solution. The concentrations of suspended AlphaSan in the replicate test beakers within the same

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chamber were not measured. Thus, the variation in AlphaSan among the four beakers in each test chamber cannot be determined as recommended by OPPTS Guideline 850.1010. However, given the insoluble nature of the test substance, we believe the laboratory's test chamber design was appropriate and necessary.

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> Preferred species is <i>Daphnia magna</i>	<i>Daphnia magna</i>
All organisms are approximately the same size and weight?	Not reported
<u>Life Stage</u> Daphnids: 1 <sup>st</sup> instar (< 24 hr) Amphipods, stoneflies, and mayflies: 2 <sup>nd</sup> instar Midges: 2 <sup>nd</sup> & 3 <sup>th</sup> instar	1 <sup>st</sup> instar (≤ 24 hr)
<u>Supplier</u>	Wildlife International Ltd. cultures
All organisms from the same source?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
<u>Acclimation Period</u> Minimum 7 days	Neonates were used and were less than 24 hours old. Adult daphnids were cultured in well water at the same temperature as used during the test.
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	Adults showed no signs of stress during the holding period.
If treated for disease, was there no sign of the disease remaining during the 48 hr prior to testing?	Not reported
<u>Feeding</u> No feeding during the study	No feeding during the study

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Guideline Criteria	Reported Information
<u>Pretest Mortality</u> No more than 3% mortality 48 hr prior to testing	Not reported

C. Test System

Guideline Criteria	Reported Information
<u>Source of dilution water</u> Soft reconstituted water or water from a natural source, <i>not</i> dechlorinated tap water	Well water, passed through a sand filter (pore size 25 $\mu\text{m}$ ) and aerated in storage tank. Prior to use, filtered to 0.45 $\mu\text{m}$ to remove microorganisms. Water then diluted with reverse osmosis-treated water to reduce hardness to between 50 and 60 mg/L as $\text{CaCO}_3$ .
Does water support test animals without observable signs of stress?	Not reported
<u>Water Temperature</u> Daphnia: 20 °C; amphipods 17 °C; midges and mayflies 22 °C; stoneflies: 12 °C	20.4 - 20.6 °C
pH Prefer 7.2 to 7.6	7.9 - 8.3
<u>Dissolved Oxygen</u> Static: $\geq 60\%$ during 1 <sup>st</sup> 48 hr and $\geq 40\%$ during 2 <sup>nd</sup> 48 hr, flow-through: $\geq 60\%$	$> 8.6$ mg/L ( $> 96\%$ of saturation for entire test period)
<u>Total Hardness</u> Prefer 40 to 48 mg/L as $\text{CaCO}_3$	56 mg/L as $\text{CaCO}_3$ (dilution water measurement)
<u>Test Aquaria</u> 1. Material: Glass or stainless steel 2. Size: 250 mL (daphnids and midges) or 3.9 L 3. Fill volume: 200 mL (daphnids and midges) or 2-3 L	1. Material: glass aquaria, with glass beakers placed inside. 2. Size: 38 L aquaria, each with four 300 mL beakers for the daphnids with screens on the side to allow for water exchange with the aquarium. 3. 10 L in aquaria; water depth in each beaker 8.0 cm. Volume in each beaker not reported.
<u>Type of Dilution System</u> Must provide reproducible supply of toxicant	Static Test: Appropriate quantities of a primary stock suspension was added to the dilution water (in the vortex created by magnetic stir bars) in the test chambers, and mixing continued for 30 min.

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Guideline Criteria	Reported Information
<b>Flow Rate</b> Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	N/A
<b>Biomass Loading Rate</b> Static: $\leq 0.8$ g/L at $\leq 17^\circ\text{C}$ , $\leq 0.5$ g/L at $> 17^\circ\text{C}$ ; flow-through: $\leq 1$ g/L/day	N/A
<b>Photoperiod</b> 16 hr light, 8 hr dark	16 hr light, 8 hr dark
<b>Solvents</b> Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests	None

D. Test Design

Guideline Criteria	Reported Information
<b>Range Finding Test</b> If $LC_{50} > 100$ mg/L, then no definitive test is required	Yes: 0% mortality at 10 $\mu\text{g/L}$ (nominal), 60% at 100 $\mu\text{g/L}$ , and 100% at 1000 $\mu\text{g/L}$ .
<b>Nominal Concentrations of Definitive Test</b> Control & 5 treatment levels; a geometric series with each concentration being at least 60% of the next higher one	Control and 5 nominal treatment levels ( $\mu\text{g/L}$ ): of 20, 40, 80, 160, 320 and control. Each concentration is 50% of the next higher treatment (a geometric series), consistent with OPPTS Guideline 850.1010.
<b>Number of Test Organisms</b> Minimum 20/level, may be divided among containers	20/level (5 organisms per beaker; 4 beakers per aquarium)
Test organisms randomly or impartially assigned to test vessels?	Yes
<b>Water Parameter Measurements</b> 1. Temperature: Measured continuously or, if water baths are used, every 6 hr, may not vary $> 1^\circ\text{C}$ 2. DO and pH: Measured at beginning of test and every 48 hr in the high, medium, and low doses and in the control	Temperature, DO, and pH were measured at the beginning and end of the test. Temperature was measured continuously over approximately the first 12 hours in the control test chamber.

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<p><b>Chemical Analysis</b> Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used</p>	<p>Yes. AlphaSan was not soluble, although its active ingredient, silver, was soluble. To keep AlphaSan in suspension, test chambers were continuously mixed with magnetic stir bars and plates throughout the experiment. The test solutions were clear and colorless when checked at 0, 24, and 48 hrs. See Reviewer's Comments (Section 14) for description of analytic results.</p>
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Comments:

Total silver and zirconium determinations were made using inductively coupled plasma atomic emission spectrometry (ICP-AES). First, samples of the test solutions were dried and digested with concentrated hydrofluoric and 50% nitric acid. These were brought to volume with dilution solvent and placed in calibrated 50 mL centrifuge tubes and sent for ICP-AES. Emission was monitored at wavelengths of 328.068 and 338.289 nm.

Dissolved silver and zirconium determinations were made using ICP-AES configured for ultrasonic nebulization sample introduction. First, samples of the test solutions were filtered through a 0.2µm x 13 mm pore filter into the centrifuge tubes. Emission was monitored at wavelengths of 339.197 and 343.823 nm.

Calibration standards were prepared for both silver and zirconium.

12. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
<u>Control Mortality</u> Static: ≤ 10% Flow-through: ≤ 5%	0%
Percent Recovery of Chemical	Declined over time; see Reviewer's Comments in Section 14.
Raw data included?	Yes

Mortality

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Concentration ( $\mu\text{g/L}$ )		Number of Organisms	Cumulative Number Dead		
Nominal	Mean Measured (based on Ag)		Hour of Study		
			22	24	48
Control	-	20	0	0	0
Solvent Control	N/A	N/A	N/A	N/A	N/A
20	15	20	0	0	0
40	18	20	0	0	4
80	42	20	3	8	20
160	93	20	20	20	20
320	158	20	20	20	20

Other significant results: Some of the surviving daphnids in the 18  $\mu\text{g/L}$  group appeared lethargic. No daphnids at the lower exposure level (15  $\mu\text{g/L}$ ) appeared to be lethargic.

### B. Statistical Results

Method: The EC<sub>50</sub> and its 95% confidence interval for mortality and immobility (one endpoint) was calculated using the binomial method in the EPA computer program developed by C.E. Stephan. The NOEC was determined by visual inspection of the data.

48-hr EC<sub>50</sub>: 23  $\mu\text{g/L}$   
 Probit Slope: N/A

95% C.I.: 18-42  $\mu\text{g/L}$   
 NOEC: 15  $\mu\text{g/L}$

### 13. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Binomial Test EC <sub>50</sub> (95% C.I.)	Not used
Moving Average Angle EC <sub>50</sub> (95% C.I.)	Not used
Probit EC <sub>50</sub> (95% C.I.)	Data do not support probit analysis
Probit Slope	Data do not support probit analysis
NOEC (Dunnett's test)	15 $\mu\text{g/L}$ AlphaSan
Spearman-Karber 48-hr EC <sub>50</sub> (95% C.I.)	27.3 (24.9 - 29.7) $\mu\text{g/L}$ AlphaSan

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24-hr EC<sub>50</sub>: 52.5 µg/L  
48-hr EC<sub>50</sub>: 27.3 µg/L  
NOEC: 15 µg/L

95% C.I.: 44-61 µg/L  
95% C.I.: 24.9-29.7 µg/L  
Probit Slope: N/A

The authors relied on statistical software made available by EPA in 1978. We used the Spearman-Kärber method as implemented by TOXSTAT, Version 3.5 (WEST Inc, 1996) to estimate the EC<sub>50</sub>. The Spearman-Kärber test is the most appropriate given the data (i.e., one partial mortality and zero mortality in the lowest concentration and 100% mortality at the highest concentration tested). We did not log-transform the concentration data. The result is similar to the value obtained by the authors using the binomial method; however, the 95% C.I. is much smaller.

#### 14. REVIEWER'S COMMENTS

##### C. Measured Concentrations: Homogeneity and Stability over Time

The authors selected silver (Ag) and zirconium (Zr) to represent the test substance due to the insolubility of the polymeric backbone and ion-exchange properties of AlphaSan RC 2000. Results of the analyses for total and dissolved silver are presented in Tables 1 and 2 below. Results of the analyses for total and dissolved zirconium are presented in Tables 3 and 4 below.

Concentrations of total silver and zirconium declined over time. As indicated in Table 1, the concentration of total silver declined from 74.5% - 102% of nominal at test initiation, to 21.7% - 38.3% of nominal at 24 hours, reaching 16.4% - 18.3% of nominal at 48 hours. As indicated in Table 3, the concentration of total zirconium declined from 96.1% - 109% at test initiation to 21.6% - 34.0% of nominal at 24 hours, reaching 11.7% - 18.1% of nominal at 48 hours. The authors attributed the decline with time of total silver and zirconium in suspension to the molecules of AlphaSan falling out of suspension. While the laboratory attempted to minimize this loss by using magnetic stirrers, the stir rates were limited to levels that the daphnia could tolerate, as demonstrated in the earlier range-finding experiment.

Concentrations of dissolved silver exhibited a different pattern. Where quantified, dissolved silver concentrations increased somewhat over time (Table 2). The authors attributed the increase in dissolved silver from 9% - 11% of nominal to 20% - 22% of nominal between 0 and 24 hr to its continuing release from the AlphaSan ion-exchange polymer. No dissolved zirconium was detected in any sample. That result was expected because zirconium is part of the polymeric ion exchange backbone of the AlphaSan.

The silver ions in solution represent the active ingredient of AlphaSan.

Table 1. Measured Concentrations of Total Silver<sup>1</sup>

Nominal Concentrations (µg/L)	Ag Measured (µg/L)	Measured as % of Nominal
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MRID No.: 452523-02

AlphaSan	Total Ag <sup>2</sup>	0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
20	1.96	1.46	< LOQ	< LOQ	74.5	-	-
40	3.92	3.24	1.29	0.72	82.4	32.7	18.3
80	7.84	8.02	3.00	1.29	102	38.3	16.4
160	15.7	13.0	5.12	--	83.1	32.6	-
320	31.4	24.3	6.80	--	77.3	21.7	-

<sup>1</sup>Data from Table 2 of Study Report.

<sup>2</sup>Calculated based on 9.81% (w/w) Ag in AlphaSan RC 2000.

Abbr: LOQ = limit of quantitation; 0.500µg Ag/L.

- Not measured due to 100% mortality of daphnids.

**Table 2. Measured Concentrations of Dissolved Silver<sup>1</sup>**

Nominal Concentrations (µg/L)		Ag+ Measured (µg/L)			Measured as % of Nominal Dissolved Ag+		
AlphaSan	Dissolved Ag <sup>2</sup>	0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
20	1.96	< LOQ	< LOQ	< LOQ	-	-	-
40	3.92	< LOQ	< LOQ	< LOQ	-	-	-
80	7.84	0.604	0.835	0.784	7.70	10.6	10.0
160	15.7	1.46	3.16	-	9.31	20.1	-
320	31.4	3.38	6.78	-	10.8	21.6	-

<sup>1</sup>Data from Table 4 of Study Report

<sup>2</sup>Calculated based on 9.81% (w/w) Ag in AlphaSan RC 2000.

LOQ = limit of quantitation; 0.500µg Ag/L.

- Not measured due to 100% mortality of daphnids.

**Table 3. Measured Concentrations of Total Zirconium<sup>1</sup>**

Nominal Concentrations (µg/L)		Zr Measured (µg/L)			Measured as % of Nominal		
AlphaSan	Total Zr <sup>2</sup>	0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
20	6.62	6.67	< LOQ	< LOQ	101	-	-
40	13.2	14.4	4.50	2.39	109	34.0	18.1
80	26.5	26.7	6.56	3.09	101	24.8	11.7
160	53.0	50.9	17.0	-	96.1	32.1	-
320	106	105	22.9	-	98.7	21.6	-

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<sup>1</sup>Data from Table 3 of Study Report.

<sup>2</sup>Calculated based on 33.1% (w/w) Zr in AlphaSan RC 2000.

Abbr: LOQ = limit of quantitation; 2.00 µg Zr/L. – Not measured due to 100% mortality of daphnids.

**Table 4. Measured Concentrations of Dissolved Zirconium <sup>1</sup>**

Nominal Concentrations (µg/L)		Zr Measured (µg/L)			Measured as % of Nominal		
AlphaSan	Total Zr <sup>2</sup>	0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
20	6.62	< LOQ	< LOQ	< LOQ	–	–	–
40	13.2	< LOQ	< LOQ	< LOQ	–	–	–
80	26.5	< LOQ	< LOQ	< LOQ	--	–	–
160	53.0	< LOQ	< LOQ	< LOQ	--	–	–
320	106	< LOQ	< LOQ	< LOQ	--	–	–

<sup>1</sup>Data from Table 5 of Study Report.

<sup>2</sup>Calculated based on 33.1% (w/w) Zr in AlphaSan RC 2000.

Abbr: LOQ = limit of quantitation; 2.00 µg Zr/L. – Not measured due to 100% mortality of daphnids.

**D. Percent Recovery**

The authors reported the results of their concurrent quality control measurements based on samples spiked with AlphaSan at 10, 100, and 500 µg/L and measured for total and dissolved silver and zirconium. For silver, the mean recovery of total silver ranged from 79% to 82% and the mean recovery of the dissolved metal ranged between 65% and 74% (Table 5 below) for the three spiked concentrations tested. For zirconium, the mean recovery of total zirconium from spiked samples ranged from 87% to 105% and the mean recovery of dissolved zirconium ranged from 53% to 103% (Table 6 below). The lowest recovery value of 53% of the dissolved zirconium occurred in the sample spiked at a concentration of 500 µg/L AlphaSan (estimated 166 µg/L zirconium). The authors expected these results based on the structure of the test substance, which consists of a polymeric ion exchange backbone with relatively low water solubility. Particularly at higher concentrations, molecules of this size were not expected to pass quantitatively through the 0.20 µm pore filter during the analysis of dissolved samples.

The authors did not calculate standard deviations or coefficients of variation (CVs) for the recovery data, so our reviewers did. Table 5 indicates that measurements of total silver, particularly in the lowest exposure concentration of AlphaSan (10 µg/L), were not very precise, with a coefficients of variation (CVs) in excess of 37% for the nominal concentration of 0.981 µg/L and CVs of 13% to 16% for the nominal concentrations of 9.81 and 49.1 µg/L silver, respectively. Table 6 indicates that measurements of total zirconium, on the other hand, were reasonably precise, with CVs of between 3% to 8% over the same range of AlphaSan concentrations (i.e., 10, 100, and 500 µg/L). The concentrations of silver and zirconium in the spiked samples at the lowest concentration

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of AlphaSan analyzed (i.e., 10 µg/L) were close to the laboratory's limits of quantitation for both silver (0.500 µg/L) and zirconium (2.0 µg Zr/L).

Table 5 shows CVs for measurements of dissolved silver that range between 11% and 22%. Table 6 shows CVs for measurements of dissolved zirconium that range between 5% and 24%. These are high, but acceptable.

**Table 5. Recovery of Silver in Quality Control Samples<sup>1</sup>**

Nominal Concentration (µg/L)		Measured Concentration (µg Ag/L) <sup>2</sup> (sample number)				Summary Statistics (µg Ag/L)		
Alpha-San	Ag <sup>3</sup>	(1)	(2)	(3)	(4)	Mean (% Recovery)	Standard Deviation	Coefficient of Variation
Total Silver								
10	0.981	1.14	0.762	0.534	<LOQ	0.812 (81.2%)	0.306 (N=3)	37.7%
100	9.81	7.81	7.44	7.24	10.1	8.15 (81.5%)	1.32 (N=4)	16.2%
500	49.1	34.1	34.3	44.9	41.0	38.6 (78.6 %)	5.30 (N=4)	13.7%
Dissolved Silver								
10	0.981	0.581	0.605	0.715	–	0.634 (64.6%)	0.0714 (N=3)	11.3%
100	9.81	8.11	5.78	5.96	–	6.62 (67.5%)	1.30 (N=3)	19.6%
500	49.1	45.3	29.3	34.9	–	36.5 (74.3%)	8.12 (N=3)	22.2%

<sup>1</sup>Data from Appendix 3.9 of Study Report; mean, SD, and CV calculated by reviewers.

<sup>2</sup>In each of the samples measured, up to a total of four samples.

<sup>3</sup>Calculated based on 9.81% (w/w) Ag in AlphaSan RC 2000.

**Table 6. Recovery of Zirconium in Quality Control Samples<sup>1</sup>**

Nominal Concentration (µg/L)		Measured Concentration (µg Zr/L) <sup>2</sup> (sample number)				Summary Statistics (µg Zr/L)		
Alpha-San	Zr <sup>3</sup>	(1)	(2)	(3)	(4)	Mean (% Recovery)	Standard Deviation (N)	Coefficient of Variation
Total Zirconium								
10	3.31	3.86	3.42	3.32	3.26	3.47 (105%)	0.271 (N=4)	7.81%
100	33.1	31.2	28.3	28.4	30.0	29.5 (89.1%)	1.39 (N=4)	4.71%
500	166	150	141	152	145	147 (88.6%)	4.97 (N=4)	3.39%

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Dissolved Zirconium								
10	3.31	3.41	3.25	3.59	–	3.42 (103%)	0.170 (N=3)	4.97%
100	33.1	22.4	25.0	33.7	–	27.0 (81.6%)	5.29 (N=3)	19.6%
500	166	113	79.4	73.3	–	88.6 (53.4%)	21.4 (N=3)	24.2%

<sup>1</sup>Data from Appendix 3.10 of Study Report; mean, SD, and CV calculated by reviewers.

<sup>2</sup>In each of the samples measured, up to a total of four samples.

<sup>3</sup>Calculated based on 33.1% (w/w) Zr in AlphaSan RC 2000.

**C. Conclusions:** Although the measurements of the active ingredient, dissolved silver ions, in the test solutions were below the detection limit at the LOEC of 18  $\mu\text{g/L}$  AlphaSan, the authors appear to have taken all reasonable approaches to measuring the concentration of the test substance, a polymeric ion-exchange substance, and the active ingredient in the test solutions. Although the AlphaSan appears to have slowly settled out of solution, again, the authors took all reasonable steps to attempt to keep it suspended and to measure its loss from the water column over time. They also used the mean measured values over the duration of the experiment to estimate the  $\text{EC}_{50}$ , as is appropriate. Thus, we conclude that the difficulties identified in this study are inherent in the properties of the test material itself in an aqueous medium. We therefore classify this test as core.