

# ANIMAL HEALTH LABORATORIES LABORATORY REPORT



Department of Agriculture and Food  
AGWEST Animal Health Laboratories



3 Baron-Hay Court South Perth, WA 6151 • Tel: (08) 9368 3351 • Fax: (08) 9474 1881  
444 Albany Highway, Albany, WA 6330 • Tel: (08) 9892 8444 • Fax: (08) 9892 8564

Case Number: AS-10-3341-F-V1

Final Report

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Date: 16-NOV-2010

Your Ref: Not Supplied

Enquiries: Dr Nicky Buller(Bacteriology Perth)

To: Gavin Partridge  
Challenger TAFE  
1 Fleet St  
Fremantle  
WA

cc.

Owner:

Project: Animal sample testing - non disease investigation

Species: Water - Water

Samples Received: 18 water samples

Date Collected: Not Supplied

Date Received: 27-OCT-2010

Submission Number: 71347

## Methods

Two experiments were conducted to determine the concentration of chlorine dioxide (CleanOxide) required to kill the fish pathogen, *Streptococcus iniae*. The Australian Centre for Applied Aquaculture Research (ACAAR) prepared a stock solution of chlorine dioxide (CleanOxide) for each experiment and provided it to the Animal Health Laboratories. A 7500 ppm stock solution was prepared according to the manufacturers directions and diluted to achieve a working solution of 2000 ppb and 12000 ppb for trial 1 and 2, respectively. The concentration of this stock solution was confirmed spectrophotometrically immediately prior to use in the disinfection trials using a Hach DR 2400 with Method #10126.

Disinfection trials were conducted at the The Animal Health Laboratories. Laboratory staff prepared a pure culture of the pathogen *S. iniae* in nutrient broth at a concentration of  $\sim 2 \times 10^5$  CFU/mL. Prior to disinfection trials, bacteria was concentrated then resuspended in isotonic saline to reduce the organic load within the vials; ensuring that CleanOxide was targeted at bacteria, rather than to oxidising the nutrients within the broth. The effectiveness of nine concentrations of CleanOxide against *S. iniae* were tested in duplicate at concentrations ranging from 0 to 347 ppb in Trial 1 and 0 to 3100 ppb in Trial 2. After exposure to the desired concentration for 60 minutes, CleanOxide was neutralised with sodium thiosulphate and the remaining bacteria enumerated.

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## Results

Bacterial counts for Trial 1 are presented in Table 1 and Figure 1 and for Trial 2 in Table 2 and Figure 2. There were no significant reductions in *S. iniae* at CleanOxide concentrations up to 347 ppb in Trial 1. In Trial 2, kill rates of >97% were obtained at a CleanOxide concentration >1127 ppb. At a concentration >1459 ppb a kill rate >99.99% was achieved. These results demonstrate that *S. iniae* is more difficult to kill than, *V. mimicus* which had was reduced by 64% and by >99.9% at CleanOxide concentrations of 332 and 369 ppb.

Table 1.

Spec No.	Spec ID	Spec Description	Total count
1	1	0 ppb ClO2	1 200 000 cfu/mL
2	2	0 ppb ClO2	840 000 cfu/mL
3	3	44 ppb ClO2	1 100 000 cfu/mL
4	4	44 ppb ClO2	1 210 000 cfu/mL
5	5	85 ppb ClO2	920 000 cfu/mL
6	6	85 ppb ClO2	1 200 000 cfu/mL
7	7	124 ppb ClO2	1 165 000 cfu/mL
8	8	124 ppb ClO2	910 000 cfu/mL
9	9	161 ppb ClO2	1 005 000 cfu/mL
10	10	161 ppb ClO2	905 000 cfu/mL
11	11	196 ppb ClO2	865 000 cfu/mL
12	12	196 ppb ClO2	1 200 000 cfu/mL
13	13	229 ppb ClO2	1 295 000 cfu/mL
14	14	229 ppb ClO2	870 000 cfu/mL
15	15	320 ppb ClO2	1 085 000 cfu/mL
16	16	320 ppb ClO2	1 120 000 cfu/mL
17	17	347 ppb ClO2	860 000 cfu/mL
18	18	347 ppb ClO2	985 000 cfu/mL

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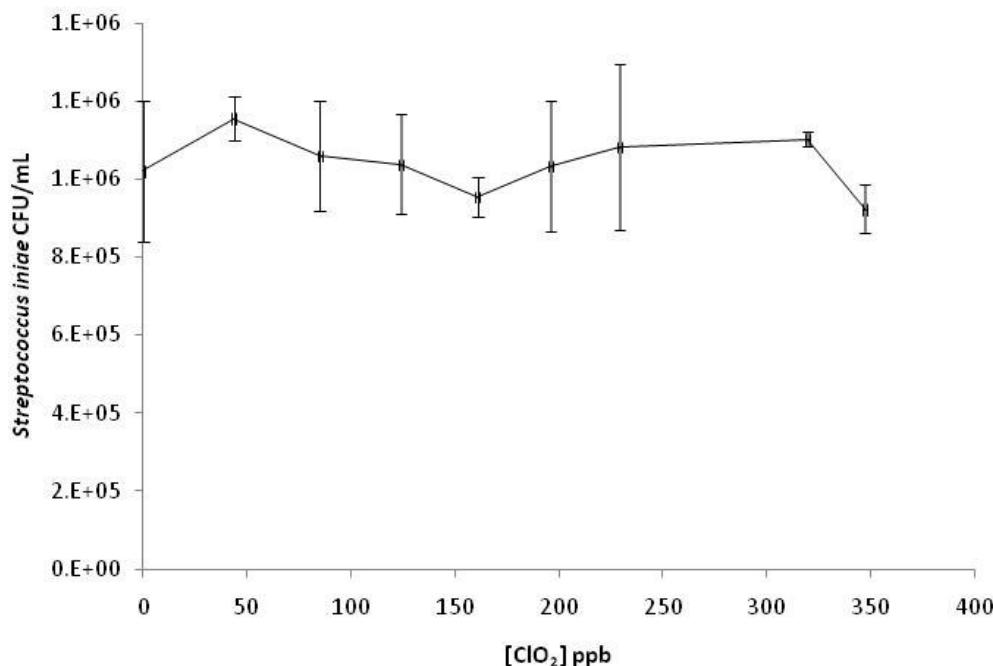


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**Figure 1**

**Table 2.**

Spec No.	Spec ID	Spec Description	Total count
1	1	0 ppb ClO2	215 000 cfu/mL
2	2	0 ppb ClO2	150 000 cfu/mL
3	3	326 ppb ClO2	99 000 cfu/mL
4	4	326 ppb ClO2	78 000 cfu/mL
5	5	361 ppb ClO2	62 000 cfu/mL
6	6	361 ppb ClO2	64 500 cfu/mL
7	7	477 ppb ClO2	42 000 cfu/mL
8	8	477 ppb ClO2	23 000 cfu/mL
9	9	590 ppb ClO2	25 500 cfu/mL
10	10	590 ppb ClO2	19 500 cfu/mL
11	11	775 ppb ClO2	23 000 cfu/mL
12	12	775 ppb ClO2	32 500 cfu/mL
13	13	1127 ppb ClO2	6000 cfu/mL
14	14	1127 ppb ClO2	5000 cfu/mL
15	15	1459 ppb ClO2	30 cfu/mL
16	16	1459 ppb ClO2	10 cfu/mL
17	17	3100 ppb ClO2	10 cfu/mL
18	18	3100 ppb ClO2	30 cfu/mL

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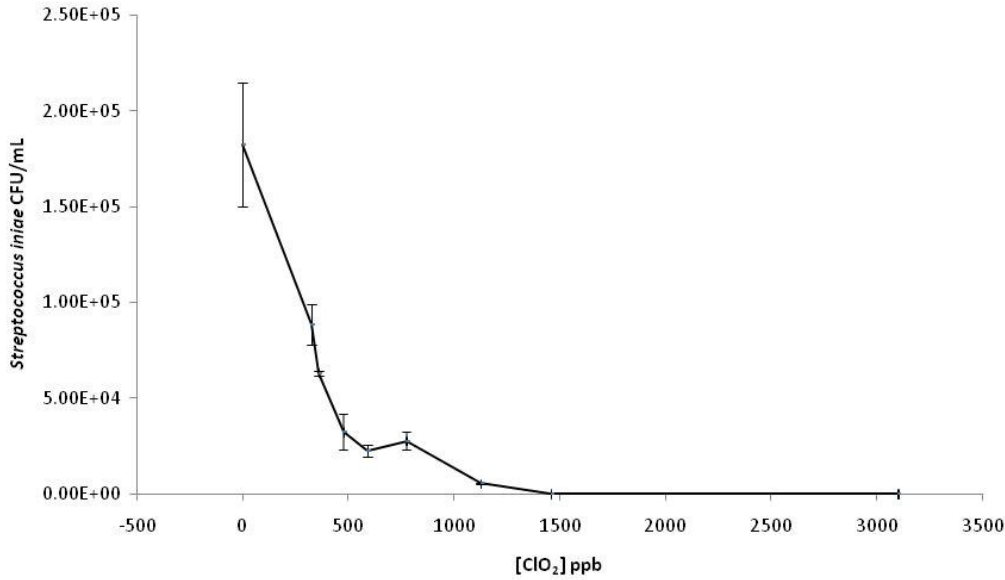


Figure 2

Yours faithfully

Dr Nicky Buller  
SENIOR MICROBIOLOGIST

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