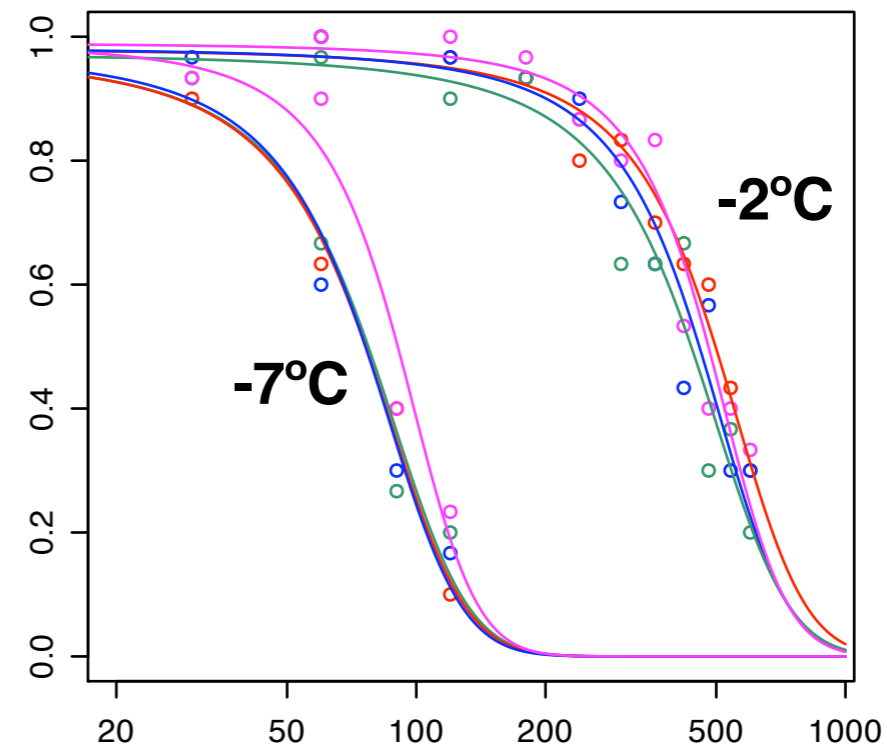
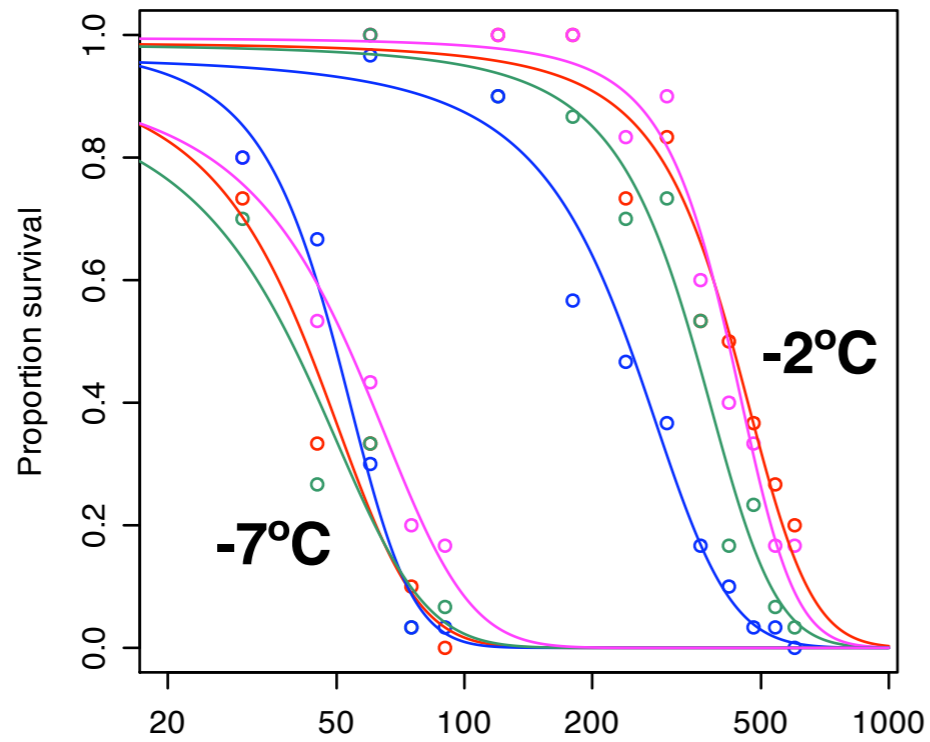


a

Oregon R



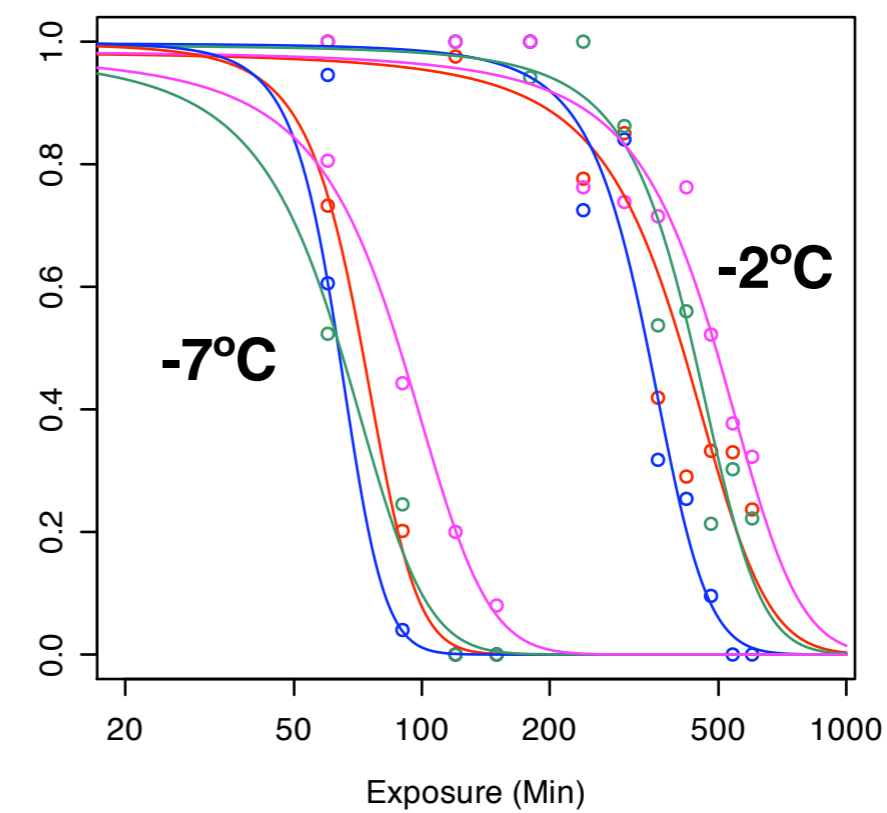
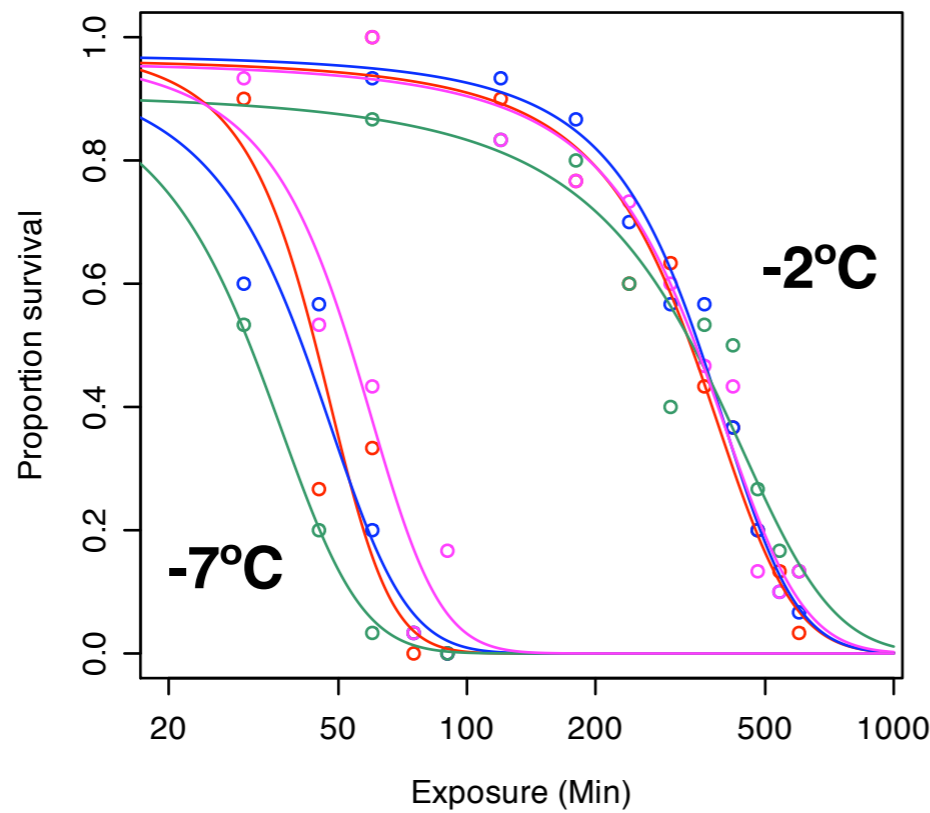
Berlin K

b

Stage I  
Stage II  
Stage III  
Stage W

c

Canton S



W5N

d

Basal cold tolerance in the larval instars of 4 strains of *D. melanogaster*. Upper right curves represent -2°C exposure & survival in 4 instars. The lower left represents -7°C tolerance in the various larval stages.

APPENDIX 1-1

**Acute cold tolerance data for four strains of *Drosophila melanogaster* and their 4 larval stages**

Cold tolerance of *Drosophila melanogaster* strain Oregon R larval stages after exposure to -7 °C for various time periods. Table contains the mean (top) and sd (bottom). The data is also presented as survival curves in appendix 1.

Exposure (min)	Larval stage			
	1	2	3	4
30	.7333333	.8	.7	.8
	.1527525	.1	.2	.2
45	.3333333	.6666667	.2666667	.5333333
	.4163332	.2886751	.057735	.1527525
60	.3333333	.3	.3333333	.4333333
	.3511885	.1	.3055051	.321455
75	.1	.0333333	.0333333	.2
	.1732051	.057735	.057735	.1
90	0	.0333333	.0666667	.1666667
	0	.057735	.1154701	.2886751
120				
150				

Cold tolerance of *Drosophila melanogaster* strain Canton S larval stages after exposure to -7 °C for various time periods. Table contains the mean (top) and sd (bottom). The data is also presented as survival curves in appendix 1.

Exposure (min)	Larval stage			
	1	2	3	4
30	.9	.6	.5333334	.9333333
	.1	.4582576	.057735	.057735
45	.2666667	.5666667	.2	.5333334
	.1527525	.2081666	0	.2081666
60	.3333333	.2	.0333333	.4333333
	.4932883	.1	.057735	.057735
75	0	.0333333	.0333333	.0333333
	0	.057735	.057735	.057735
90	0	0	0	.1666667
	0	0	0	.2886751
120				
150				

Cold tolerance of *Drosophila melanogaster* strain Berlin K larval stages after exposure to -7 °C for various time periods. Table contains the mean (top) and sd (bottom). The data is also presented as survival curves in appendix 1.

Exposure (min)	Larval stage			
	1	2	3	4
30	.9 0	.9666666 .057735	.9333333 .057735	.9333333 .057735
45				
60	.6333333 .057735	.6 .1	.6666666 .2516611	.9 .1
75				
90	.4 .1	.3 0	.2666667 .1527525	.4 .1
120	.1 .1	.1666667 .057735	.2 .1	.2333333 .057735
150				

Cold tolerance of *Drosophila melanogaster* strain W5N larval stages after exposure to -7 °C for various time periods. Table contains the mean (top) and sd (bottom). The data is also presented as survival curves in appendix 1.

Exposure (min)	Larval stage			
	1	2	3	4
30				
45				
60	7.323333 .8200205	6.056667 .5814062	5.233333 .2516612	8.056666 .3728716
75				
90	2.016667 .7751344	.4 .3605551	2.45 .9836158	4.426667 1.967901
120	0 0	0 0	0 0	2 .7
150	0 0	0 0	0 0	.8 .3605551

APPENDIX 1-2

**Chronic cold tolerance data for four strains of *Drosophila melanogaster* and their 4 larval stages**

Cold tolerance of *Drosophila melanogaster* strain Oregon R larval stages after exposure to -2 °C for various time periods. Table contains the mean (top) and sd (bottom). The data is also presented as survival curves in appendix 1.

Exposure (min)	Larval stage			
	1	2	3	4
0	1 0	1 0	1 0	1 0
60	1 0	1 0	1 0	1 0
120	.6666667 .3444803	.75 .1870829	.5833333 .3188521	.7833333 .2136976
240	.7166667 .2562551	.6333333 .2875181	.5333334 .332666	.6333333 .2804758
360	.5 .3162278	.6 .1414214	.45 .3563706	.3666667 .273252
480	.45 .3209361	.5 .363318	.2666667 .233809	.4166667 .271416
600	.35 .3331666	.5166667 .2857738	.25 .2949576	.45 .3082207
720	.1833333 .147196	.1333333 .136626	.15 .1048809	.25 .1048809
840	.05 .083666	.1 .0894427	.0833333 .132916	.1166667 .1169045
960	.0166667 .0408248	.05 .083666	.0166667 .0408248	.0166667 .0408248

Cold tolerance of *Drosophila melanogaster* strain Canton S larval stages after exposure to -2 °C for various time periods. Table contains the mean (top) and sd (bottom). The data is also presented as survival curves in appendix 1.

Exposure (min)	Larval stage			
	1	2	3	4
0	1 0	1 0	1 0	1 0
60	1 0	1 0	1 0	1 0
120	.72 .2280351	.45 .1870829	.4 .2607681	.65 .3331666
240	.6333333 .301109	.45 .2810694	.4166667 .2562551	.4666667 .242212
360	.75 .2949576	.2 .244949	.25 .2167948	.4166667 .3060501
480	.4 .1414214	.0833333 .1602082	.1666667 .1861899	.2666667 .1966384
600	.3333333 .301109	.15 .1048809	.1333333 .175119	.15 .1974842
720	.15 .1974842	.05 .083666	.05 .083666	.0833333 .1169045
840	.35 .1870829	.0166667 .0408248	.0166667 .0408248	.0333333 .0516398
960	.2833333 .3656045	.0166667 .0408248	0 0	.05 .083666

Cold tolerance of *Drosophila melanogaster* strain Berlin K larval stages after exposure to -2 °C for various time periods. Table contains the mean (top) and sd (bottom). The data is also presented as survival curves in appendix 1.

Exposure (min)	Larval stage			
	1	2	3	4
0	1 0	1 0	1 0	1 0
60	1 0	1 0	1 0	1 0
120	.8166667 .1722401	.75 .250998	.8166667 .1722401	.8666667 .2065591
240	.85 .1378405	.7 .1788854	.8666667 .175119	.8333333 .1505545
360	.9 .0894427	.6 .3162278	.65 .1760682	.7166666 .2316607
480	.6 .2366432	.5166667 .2401388	.5166667 .2316607	.6 .2280351
600	.5666667 .1032796	.4666667 .301109	.35 .083666	.5666667 .2804758
720	.4166667 .2786874	.1 .1264911	.0666667 .0816497	.35 .2258318
840	.5833333 .3600926	.15 .1048809	.1 .1264911	.1166667 .1602082
960	.25 .3781534	.1333333 .1966384	.0166667 .0408248	.0833333 .1169045



Cold tolerance of *Drosophila melanogaster* strain W5N larval stages after exposure to -2 °C for various time periods. Table contains the mean (top) and sd (bottom). The data is also presented as survival curves in appendix 1.

Exposure (min)	Larval stage			
	1	2	3	4
0	1 0	1 0	1 0	1 0
60	1 0	1 0	1 0	1 0
120	.8 .303315	.85 .1870829	.75 .2738613	.8833333 .1602082
240	.7333333 .233809	.4166667 .4262237	.6666667 .3011109	.8333333 .2250926
360	.8 .167332	.4 .3521363	.6 .3577709	.6 .3405877
480	.5333334 .2160247	.4333333 .2875181	.6 .2683281	.45 .250998
600	.4666667 .2065591	.5166667 .3060501	.35 .2738613	.4 .2366432
720	.3833333 .3371449	.1666667 .0816497	.15 .1224745	.1333333 .2804758
840	.5833333 .3311596	.1166667 .147196	.0833333 .1169045	.1333333 .121106
960	.1333333 .121106	.0166667 .0408248	.05 .083666	0 0

## APPENDIX 2

### Effects of RCH on acute and chronic cold tolerance in four larval stages in four different strains of *Drosophila melanogaster*

#### Effect of RCH on acute cold tolerance

##### Oregon R

stage	Without RCH	-sd	With RCH	+sd
1	0.1	0.1	0.33	0.29
2	0.03	0.06	0.13	0.06
3	0.1	0	0.4	0.2
w	0.2	0.1	0.47	0.35

##### Berlin K

stage	Without RCH	-sd	With RCH	+sd
1	0.07	0.07	0.13	0.12
2	0.13	0.15	0.27	0.15
3	0.03	0.06	0.47	0.12
w	0.03	0.06	0.47	0.35

##### Canton S

stage	Without RCH	-sd	With RCH	+sd
1	0.03	0.03	0.81	0.03
2	0.12	0.08	0.39	0.07
3	0.06	0.02	0.76	0.03
w	0.35	0.13	0.59	0.08

##### W5N

stage	Without RCH	-sd	With RCH	+sd
1	0.38	0.12	0.69	0.03
2	0.086	0.03	0.4	0.04
3	0.42	0.13	0.52	0.001
w	0.72	0.23	0.88	0.05

## Effect of RCH on chronic cold tolerance

### Oregon R

stage	Without RCH	-sd	With RCH	+sd
1	0.16	0.05	0.75	0.03
2	0.1	0.04	0.13	0.08
3	0.35	0.09	0.81	0.13
w	0.89	0.09	0.84	0.02

### Berlin K

stage	Without RCH	-sd	With RCH	+sd
1	0.03	0.01	0.4	0.1
2	0.33	0.28	0.13	0.15
3	0.03	0.01	0.27	0.12
w	0.03	0.01	0.37	0.06

### Canton S

stage	Without RCH	-sd	With RCH	+sd
1	0.2	0.02	0.67	0.12
2	0.24	0.05	0.64	0.04
3	0.11	0.01	0.52	0.20
w	0.08	0.02	0.37	0.09

### W5N

stage	Without RCH	-sd	With RCH	+sd
1	0.04	0.01	0.44	0.03
2	0.01	0.002	0.34	0.08
3	0.04	0.01	0.50	0.13
w	0.07	0.02	0.6	0.02

## APPENDIX 3

### Effect of heat shock on acute and chronic cold tolerance in four strains of the four larval stages of *Drosophila melanogaster*

#### Acute cold tolerance after heat shock

##### Oregon R

stage	Without RCH	-sd	With RCH	+sd
1	0.1	0.052	0.07	0.03
2	0	0	0.09	0.02
3	0.16	0.03	0.24	0.07
w	0.39	0.11	0.21	0.03

##### Canton S

stage	Without RCH	-sd	With RCH	+sd
1	0.15	0.05	0	0
2	0.13	0.05	0.07	0.02
3	0.18	0.05	0.19	0.09
w	0.42	0.07	0.18	0.06

##### Berlin K

stage	Without RCH	-sd	With RCH	+sd
1	0.17	0.05	0.25	0.08
2	0.19	0.07	0.06	0.02
3	0.27	0.03	0.44	0.04
w	0.19	0.04	0.78	0.09

##### W5N

stage	Without RCH	-sd	With RCH	+sd
1	0.08	0.03	0.2	0.06
2	0.07	0.02	0.15	0.04
3	0.13	0.1	0.26	0.09
w	0.12	0.04	0.24	0.07

## Chronic cold tolerance after heat shock

### Oregon R

stage	Without RCH	-sd	With RCH	+sd
1	0.09	0.04	0.08	0.03
2	0.02	0.01	0.01	0.01
3	0.1	0.03	0.07	0.03
w	0.04	0.001	0.13	0.02

### Canton S

stage	Without RCH	-sd	With RCH	+sd
1	0.1	0.03	0.04	0.02
2	0.13	0.05	0.02	0.01
3	0.08	0.04	0.1	0.03
w	0.07	0.01	0.06	0.05

### Berlin K

stage	Without RCH	-sd	With RCH	+sd
1	0.14	0.08	0.07	0.03
2	0.23	0.02	0.06	0.02
3	0.17	0.04	0.09	0.03
w	0.2	0.09	0.12	0.02

### W5N

stage	Without RCH	-sd	With RCH	+sd
1	0.08	0.01	0.09	0.02
2	0.15	0.08	0	0
3	0.12	0.13	0.22	0.05
w	0.04	0.02	0.19	0.08

APPENDIX 4

Effect of combined RCH and heat shock on acute and chronic cold tolerance in *Drosophila melanogaster* larval stages

Oregon R: Effect of RCH, HS, and combination pretreatments on acute and chronic cold exposure

Stage	Oregon R Acute		Oregon R Chronic	
	Control	sd	Control	sd
1	0.23	0.08	0.1	0.03
2	0	0	0.16	0.03
3	0.25	0.05	0.08	0.01
w	0.20	0.06	0.06	0.01

Stage	RCH		RCH	
	RCH	sd	RCH	sd
1	0.34	0	0.19	0.03
2	0.29	0	0.19	0.09
3	0.12	0.02	0.03	0.03
w	0.3	0.01	0.01	0.06

Stage	HS		HS	
	HS	sd	HS	sd
1	1	0	0.16	0.06
2	1	0	0.22	0.04
3	0.43	0.15	0.06	0.05
w	1	0	0.01	0.01

Stage	RCH+HS		RCH+HS	
	RCH+HS	sd	RCH+HS	sd
1	0.57	0.06	0.02	0.03
2	0.57	0.09	0.06	0.06
3	0.67	0.17	0	0
w	0.42	0.07	0.01	0.01

Stage	HS+RCH		HS+RCH	
	HS+RCH	sd	HS+RCH	sd
1	1	0	0.37	0.08
2	0.62	0.12	0.52	0.1
3	0.75	0.19	0.13	0.05
w	0.81	0.05	0.10	0.1

**Berlin K: Effect of RCH, HS, and combination pretreatments on acute and chronic cold exposure**

Berlin K Acute			Berlin K Chronic	
Stage	Control	sd	Control	sd
1	0.07	0.03	0	0
2	0.08	0.02	0	0
3	0.01	0.05	0.1	0
w	0.01	0.04	0	0

	RCH	sd	RCH	sd
1	0.23	0.1	0.1	0.1
2	0.07	0.02	0	0
3	0.43	0.13	0	0
w	0.17	0.08	0.1	0.06

	HS	sd	HS	sd
1	0.09	0.03	0	0
2	0.05	0.01	0	0
3	0.13	0.04	0	0
w	0.06	0.02	0	0

	HS+RT	sd
1	0	0
2	0.2	0.1
3	0.1	0.06
w	0.1	0.06

	RCH+HS	sd	RCH+HS	sd
1	0.15	0.06	0	0
2	0.07	0.02	0.1	0.06
3	0.16	0.03	0	0
w	0.19	0.06	0.1	0.06

	HS+RCH	sd	HS+RCH	sd
1	0.75	0.12	0	0
2	0.43	0.09	0.2	0.1
3	0.49	0.03	0	0
w	0.37	0.04	0	0

**Canton S: Effect of RCH, HS, and combination pretreatments on acute and chronic cold exposure**

Stage	Canton S Acute		Canton S Chronic	
	Control	sd	Control	sd
1	0.10	0.08	0.20	0.06
2	0.02	0.00	0.10	0.00
3	0.22	0.05	0.10	0.06
w	0.19	0.04	0.10	0.06

	RCH	SD	RCH	sd
1	0.23	0.07	0.10	0.15
2	0.07	0.01	0.40	0.10
3	0.48	0.03	0.20	0.06
w	0.21	0.08	0.10	0.12

	HS	sd	HS	sd
1	0.12	0.06		
2	0.09	0.03	0.10	
3	0.36	0.09	0.00	
w	0.29	0.07		

	HS+RT	sd
1	0.20	0.12
2	0.00	0.00
3	0.10	0.06
w	0.00	0.00

	RCH+HS	sd	RCH+HS	sd
1	0.29	0.05	0.00	0.00
2	0.17	0.04	0.10	0.00
3	0.38	0.05	0.00	0.00
w	0.60	0.11	0.00	0.00

	HS+RCH	sd	HS+RCH	sd
1	1.00	0.00	0.00	0.00
2	0.63	0.06	0.40	0.10
3	0.81	0.09	0.20	0.06
w	0.90	0.05	0.20	0.06



**W5N: Effect of RCH, HS, and combination pretreatments on acute and chronic cold exposure**

Stage	W5N Acute		W5N Chronic	
	Control	sd	Control	sd
<b>1</b>	0.08	0.04	0.10	0.06
<b>2</b>	0.03	0.00	0.10	0.06
<b>3</b>	0.08	0.03	0.10	0.06
<b>w</b>	0.13	0.05	0.10	0.00

Stage	RCH		RCH	
	Control	sd	Control	sd
<b>1</b>	0.20	0.07	0.10	0.10
<b>2</b>	0.20	0.03	0.10	0.06
<b>3</b>	0.27	0.07	0.10	0.15
<b>w</b>	0.20	0.10	0.10	0.06

Stage	HS		HS	
	Control	sd	Control	sd
<b>1</b>	0.02	0.01	0.00	0.00
<b>2</b>	0.00	0.00	0.00	0.00
<b>3</b>	0.10	0.03	0.10	0.06
<b>w</b>	0.08	0.02	0.00	0.00

Stage	RCH+HS		RCH+HS	
	Control	sd	Control	sd
<b>1</b>	0.18	0.06	0.10	0.06
<b>2</b>	0.08	0.03	0.00	0.00
<b>3</b>	0.10	0.03	0.00	0.00
<b>w</b>	0.12	0.02	0.10	0.06

Stage	HS+RCH		HS+RCH	
	Control	sd	Control	sd
<b>1</b>	0.51	0.17	0.10	0.10
<b>2</b>	0.47	0.05	0.40	0.06
<b>3</b>	0.50	0.10	0.20	0.10
<b>w</b>	0.74	0.01	0.00	0.00