

NOECs

Brachionus calyciflorus (Rotifera)

2010/06/27

2010/11/29

(NOECs)

(No observed effect concentrations)

Brachionus calyciflorus

(SDS-PAGE Electrophoresis)

(NOECs)

(67KDa 43KDa)

NOEC_s

67KDa

NOEC_s

43KDa

Reproductive-)

(NOECs

:

Determination of NOECs for the Most Important Toxic Substances to the Rotifer, *Brachionus Calyciflorus* Using Electrophoresis Method

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ABSTRACT

Sublethal toxicity studies were conducted with a rotifera (*Brachionus calyciflorus*) to determine the No observed effect concentrations (NOECs) of a different eight toxic substances based on the protein mass reduction. Protein mass (with molecular weight 43KDa - 67KDa) was quantified using electrophoresis method. Quantitation of protein concentrations demonstrated that toxicant stress reduced protein concentrations. The NOECs-43KDa and NOECs - 67KDa were reported. Since in vivo protein concentrations can be assessed in a short time, these results can be obtained in only a few hours, which is substantially less than traditional whole animal reproductive tests. NOECs - NOECs - 43KDa test were generally more sensitive end points than NOECs - NOECs-67KDa test and more sensitive than standard whole animal toxicity tests for only four toxicants.

Key words: Rotifera, Cladocera, Electrophoresis, NOECs, Protein concentrations.

(Arndt, 1993)

(Friberg-Jensen, *et al.*, 2003)

(Sladeczek, 1983)

()

:

Persoone, *et al.*,) (Hallbach, *et al.*, 1993)

.(1989

()

.(Snell and Burbank, 1995)

()

(NO observed effect concentrations) NOECs

(NOECs)) (

(Newsholme and start, 1993)

(BlackStock, 1994)

(Payne, *et al.*, 1987)

MFO, mixed function oxygenase

Alkaline

Burton and Lanza, 1987

phosphatase

Oklahoma ()

Glucosidase,) (glactosidase, amylase
 .
 (7-ethoxyresorufin-O-deethylation)
 Galgani and Bocquene .(Steadman, *et al.*, 1991)
 (acetylcholinesterase) 2000
Palaemon serratus (paraoxon)
Pleuronectes platessa *Scomber scomber*
 ()
 (*Daphnia longispina*)
 Janssen and Persoone,) .2002 obst
Daphnia (Hayes, 1992) (1993
 (4-methylumbelliferyl-glactoside) *magna*
 24- .48H-LC50
 Rotifera *Brachionus calyciflorus*
 Koste,) Brachionidae Ploimida Monogononta
 . (150-100) (250-200) .(1978
 Snell, *et al.*, 2003., Snell and Moffat,)
 (2004., Janssen, *et al.*, 1993a,b
 _ Phenol : NOECs .1
 _ Copper _ Cadmium _ Xylene _ Dimethylphenol
 Cypermethrin _ Diazinon _ Chloropyrifos
Brachionus calyciflorus
 NOECs-43KDa .2
 NOECs-67KDa
 NOECs-67KDa NOECs-43KDa .3

(1) *Brachionus calyciflorus*
 (2005) . 25x25x25
 10) 12
 () / $10^5 \times 5$ (2) *Chlorella sp*
 Negotta
) 22 .(1998) 30
 (54
) 8.5 ± 0.5
 Ferrando, *et al.*, 2003a;) / 12/12 ()
 .(Ferrando, *et al.*, 2006; May, 1987



Brachionus calyciflorus (1)

Chlorella sp
 Trebouxiophyceae Chlorellales Chlorellaceae
 (2) (taxonomicon, 2000) Protista
Brachionus calyciflorus

. 0.2

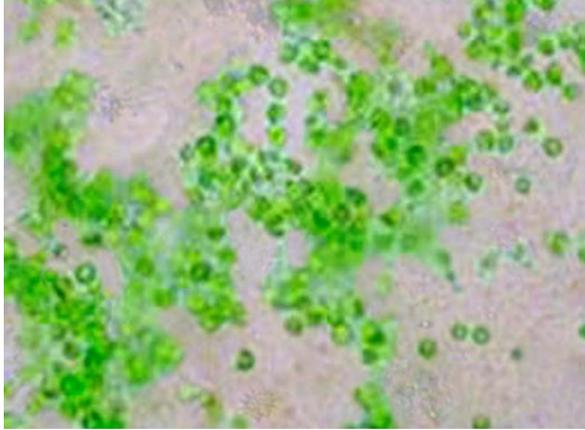
:
 (K₂PO₄ 0.250g + NH₄Cl 0.050g + FeCL₂ 0.003g + NaNO₃ 1g+
 CaCl₂ 0.058g+ MgSO₄ 0.513g)

12/12

8.5

22

.(1998) /



Chlorella sp (2)

: *Brachionus calyciflorus*

- .Dimethylphenol – Xylene – Phenol : .1
- .Cadmium - (CuCl₂) : .2
- .Chlorophenol – Diazinon – Cypermethrin : .3

Chem

Aldrich

.Sigma service

(Synthetic solution)

96mg NaHCO₃+60mg CaSO₄.2H₂O+123mg MgSO₄.7H₂O+4mg KCl)
 (

Dimethyl sulfoxide)

(DMSO

Brachionus calyciflorus

Phenol

() 36
 200) 9
 100

(Snell, *et al.*, 2003) (LC50
 24 (24

(5%) 50 . 30
 25

:) 5
 10% SDS 25% Glycerol 16mM Tris-HCl pH 6.8
 (5% β-Mercaptoethanol 0.2%

. 100
 (15%)

)
 .(Snell and Burbank, 1995) (NOEC

.
 43KDa
 NOECs-43KDa
 .NOECs-67KDa 67KDa
 (Statistica6, ANOVA, one way)

.(Walt, 2003) r

$$r = \frac{\sum (X_{1i} - X_{1}^{-}) (X_{2i} - X_{2}^{-})}{\sqrt{\sum (X_{1i} - X_{1}^{-})^2 - \sum (X_{2i} - X_{2}^{-})^2}}$$

$X_{2}^{-} = \sum X_{2i} / N$ $X_{1}^{-} = \sum X_{1i} / N$:
 . NOECs-67KDa : $X_{1i=1-8}$
 . NOECs-43KDa : $X_{2i=1-8}$
 .(8) :N

NOECs

NOECs-43KDa (43KDa)
NOECs-67KDa (67KDa)

Snell, *et al.*, 2003; Janssen, *et al.*) 24H LC50s (al.,1993b
48

) 48H Reproductive-NOECs
Snell and Moffat, 2004;) (

(Janssen, *et al.*, 1993a

(1.2-0.8)

1.2

0.8

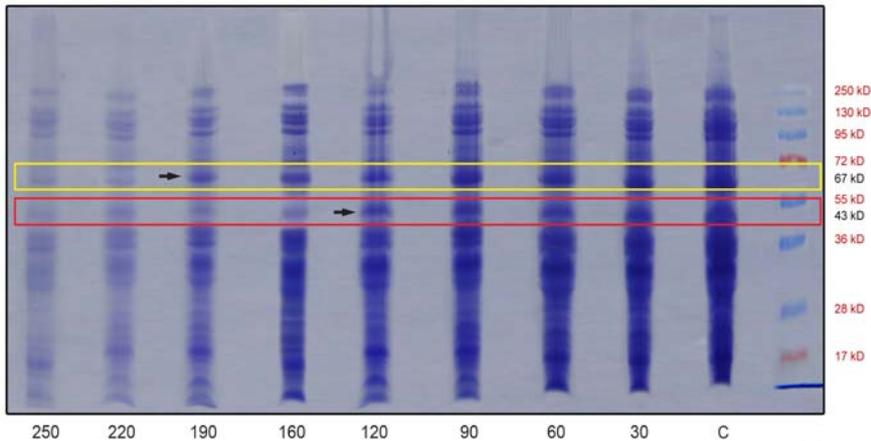
(Snell, *et al.*, 2003)

(10-3)

NOECs
(NOECs-43KDa) 43KDa

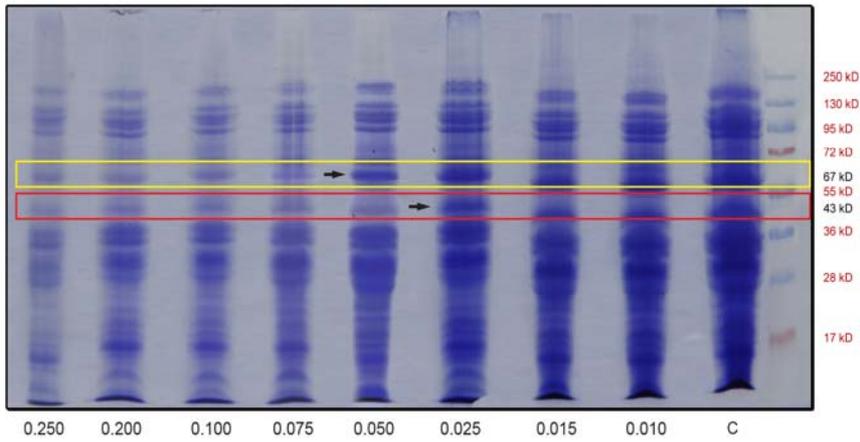
NOECs
(NOECs-67KDa) 67KDa

(C)



(3)

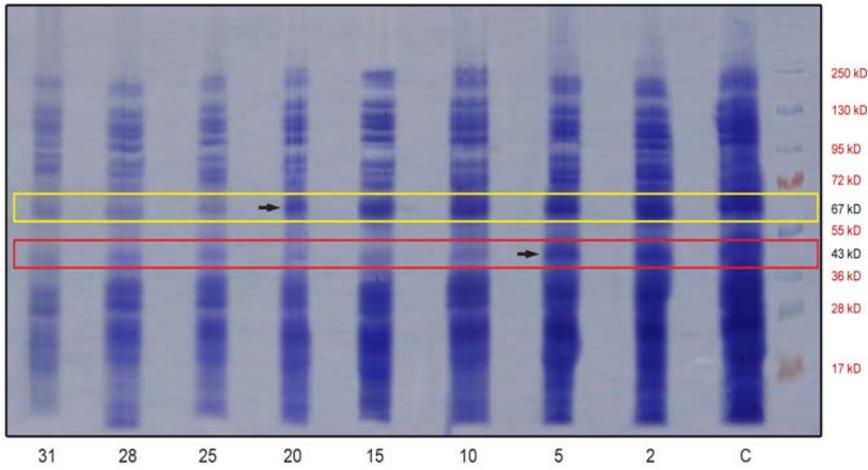
(190 120) / Xylene
(NOECs-67KDa NOECs-43KDa)



(4)

. /

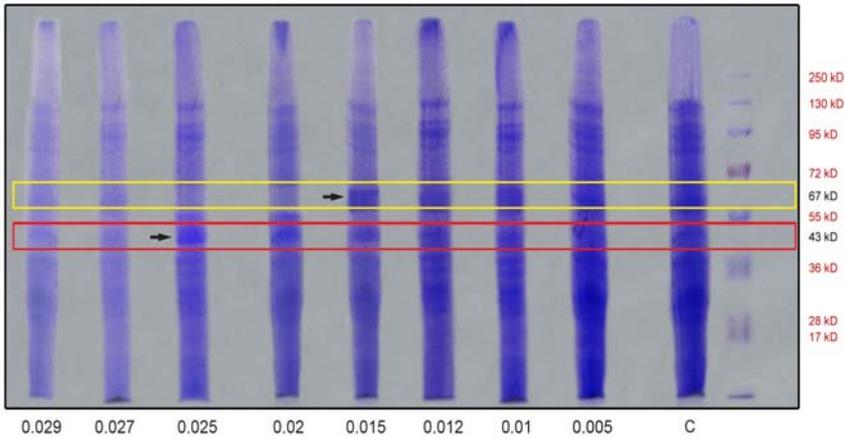
Cadmium
(NOECs-67KDa NOECs-43KDa)
(.0.050 0.025)



(5)

. /
(.20 5)

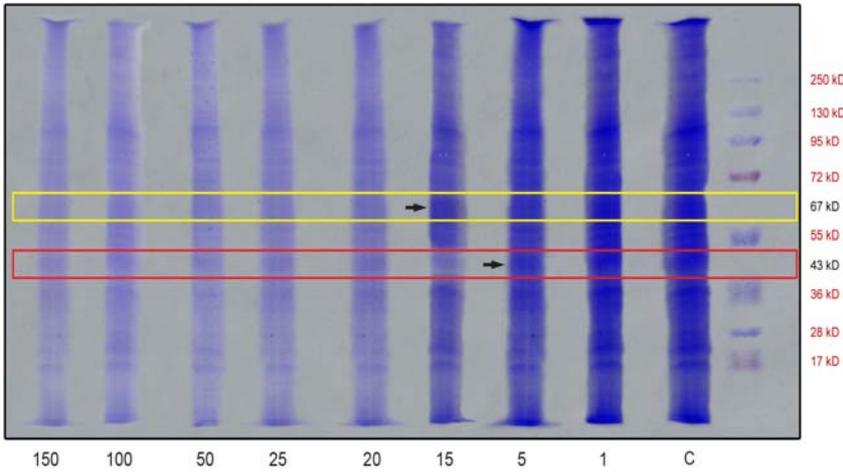
Diazinon
(NOECs-67KDa NOECs-43KDa)



(6)

. /

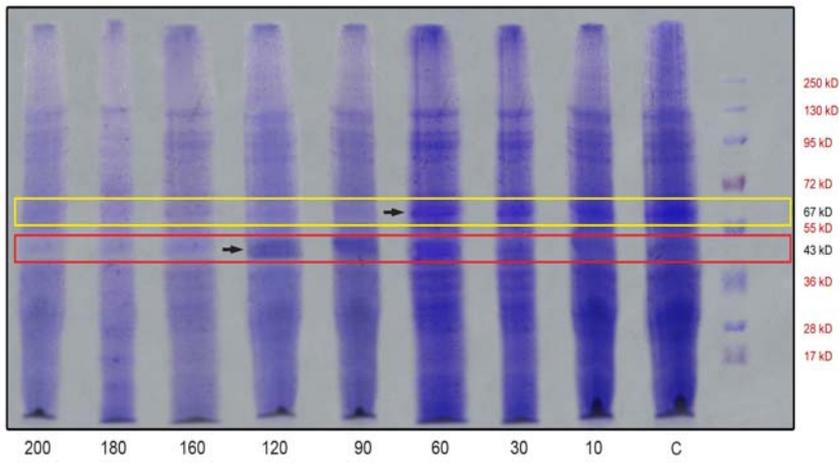
Copper
(NOECs-67KDa NOECs-43KDa)
(.0.015 0.025)



(7)

. /

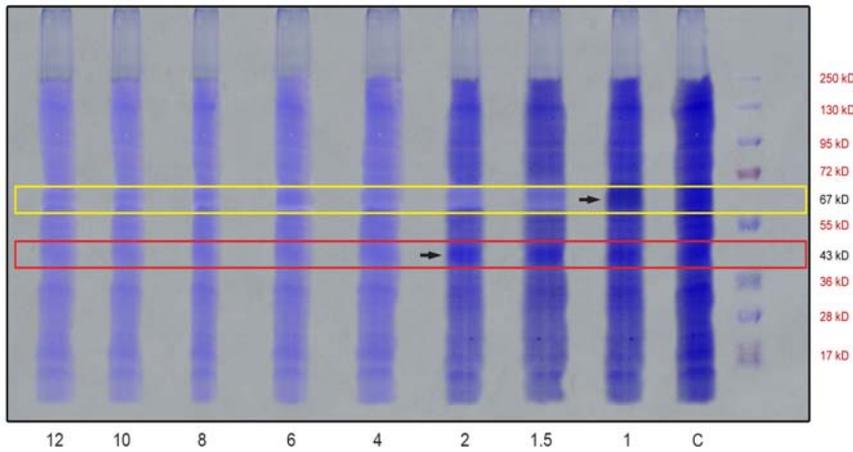
Phenol
(NOECs-67KDa NOECs-43KDa)
(.15 5)



(8)

./ **Dimethylphenol**
(NOECs-67KDa NOECs-43KDa)

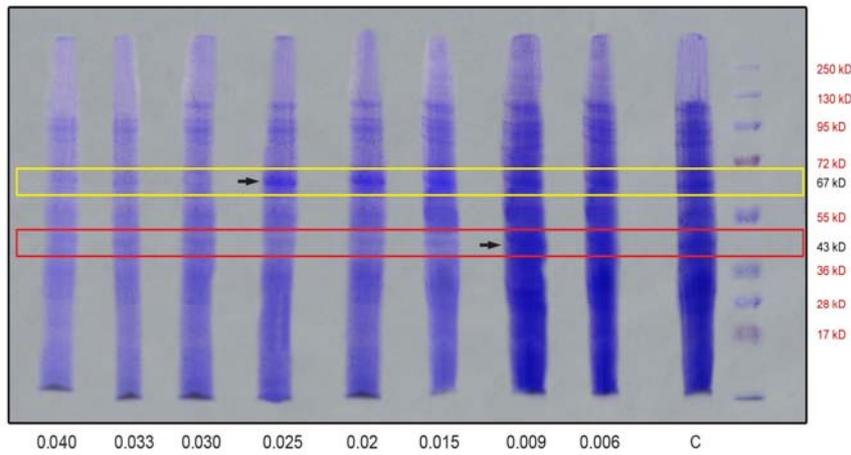
.(60 120)



(9)

./ **Chloropyrifos**
(NOECs-67KDa NOECs-43KDa)

.(1 2)



(10)

.

Cypermethrin

(NOECs-67KDa NOECs-43KDa)

.(0.025 0.009)

24H LC50s

(1)

48H Reproductive R-

(Snell, *et al.*, 2003)

(Snell and Moffat, 2004)

NOECs

() (NOECs-43KDa)

(NOECs-43KDa)

(LC50)

.(NOECs-43KDa)

(R-NOECs)

(NOECs-43KDa)

NOECs (1)

Compound	LC50	R-NOECs	NOECs-43KD _a	LC/ NOECs-43KD _a	R-NOECs/ NOECs-43KD _a
Dimethylphenol	208	2	120	1.7	0.02
Xylene	253	20	120	2.1	0.2
phenol	780	25	5	156	5
Cadmium	1.3	0.04	0.025	52	1.6
Copper	0.026	0.02	0.025	1	0.8
Cypermethrin	0.08	0.012	0.009	8.8	1.34
Chloropyrifos	12	0.23	2	6	0.1
Diazinon	31	8	5	6.2	1.6

/

R-NOECs (1)
 Xylene Dimethylphenol (NOECs-43KDa)
 Chloropyrifos
 (0.1 - 0.2 - 0.02)
 (0.8)
 R-NOECs Diazinon Cypermethrin
 1.6 (5) (NOECs-43KDa)
 .Cypermethrin 1.34
 24H LC50s (2)
 48H Reproductive R- (Snell, *et al.*, 2003)
 (Snell and Moffat, 2004) NOECs
 () (NOECs-67KDa)
 (NOECs-67KDa) (LC50)
 .(NOECs-67KDa) (R-NOECs)
 NOECs-) NOECs NOECs (2)
 (67KD_a)

Compound	LC50	R-NOECs	NOECs-67KD _a	LC/ NOECs-67KD _a	R-NOECs/ NOECs-67KD _a
Dimethylphenol	208	2	60	3.5	0.03
Xylene	253	20	190	1.3	0.11
Phenol	780	25	15	52	1.7
Cadmium	1.3	0.04	0.05	26	0.8
Copper	0.026	0.02	0.015	0. 52	1.7
Cypermethrin	0.08	0.012	0.025	3.2	0.48
Chloropyrifos	12	0.23	1	12	0.23
Diazinon	31	8	20	1.6	0.4

/
 R-NOECs 2
 Cypermethrin Xylene Dimethylphenol (NOECs-67KD_a)
 (0.48) Diazenon Chloropyrifos
 (0.03) Dimethylphenol
 (0.8)
 .(1.7)

NOECs-43KDa (3) NOECs-67KDa
 NOECs-67KDa NOECs-43KDa
 NOECs-67KDa NOECs-43KDa NOECs (3)

Compound	NOECs-43KD _a	NOECs-67KD _a	43/67
Dimethylphenol	120	60	2
Xylene	120	190	0.63
Phenol	5	15	0.33
Cadmium	0.025	0.05	0.5
Copper	0.025	0.015	1.6
Cypermethrin	0.009	0.025	0.36
Chloropyrifos	2	1	2
Diazinon	5	20	0.25

3

Diazinon Cypermethrin Cadmium Phenol Xylene (43KDa)
 (67KDa)
 (Chloropyrifos Copper Dimethylphenol)
 Correlation coefficient (r)

NOECs-43KD_a R-NOECs NOECs-67KD_a
 0.3 0.2
 NOECs- NOECs-43KDa 24H LC50s
 0.3 0.6 67KDa
 24H LC50s R-NOECs

Brachionus calyciflorus
Daphnia) :
 1H EC50s-24H EC50s- Microtox-EC50s (magna

.7days-Ceriodaphnia dubia

NOECs-43KDa (4)
Daphnia magna 24H ()
 (Janssen and Persoone, 1993) EC50s
 Janssen and Persoone,) *Daphnia magna* 1H EC50s
 McFeter,) *Microtox-EC50s* (1993
7days-Ceriodaphnia dubia (et al., 1993
 (Winner, 1988)

Brachionus NOECs-43KDa (4)

calyciflorus

(*Daphnia magna*, *Ceriodaphnia dubia*)

Toxicant	B.c/ NOECs- 43KD _a	D.m. 24H EC50	D.m/B.c	C.d. 7Day NOECs	C.d/B.c	M.t. EC50	M.t/B.c	D.m. 1h EC	D.m/B.c
Copper	0.025	0.28	11.2	0.02	0.08	39.1	1564	0.23	9.2
Cadmium	0.025	1.9	76	0.0005	0.02	489.6	19994	0.41	16.4
Phenol	5	52	10.4	4	0.8	56	11.2	37	7.4
Chloropyrifos	2	0.7	0.35	0.31	0.165			1	0.5

NOECs-67KDa (5)
Daphnia magna 24H ()
Microtox-EC50s *Daphnia magna* 1H EC50s EC50s
7days-Ceriodaphnia dubia

Brachionus NOECs-67KDa (5)

calyciflorus

(*Daphnia magna*, *Ceriodaphnia dubia*)

Toxicant	B.c/ NOECs- 67KD _a	D.m. 24H EC50	D.m/B.c	C.d. 7Day NOECs	C.d/B.c	M.t. EC50	M.t/B.c	D.m. 1h EC	D.m/B.c
Copper	0.015	8	18.6	0.02	1.4	39.1	2606	0.23	15.6
Cadmium	0.05	1.9	38	0.0005	0.01	498.6	9972	0.41	8.2
Phenol	15	52	3.5	4	0.27	56	3.7	37	2.5
Chloropyrifos	1	0.7	0.7	0.31	0.31			1	1

NOECs	(5-4)	
<i>D.m-EC50s</i>		<i>B.c/43KDa</i>
Chloropyrifos	()
<i>B.c/ NOECs-67KDa</i>		
<i>B.c/ NOECs-43KDa</i>)		
<i>7days-Ceriodaphnia dubia</i>	(<i>B.c/ NOECs-67KDa</i>	
(0.8)	<i>B.c/NOECs-43KDa</i>	
(0.02)	(0.08)	
<i>B.c/ NOECs-67KDa</i>	(0.165) Chloropyrifos	
<i>7days-Ceriodaphnia dubia</i>		
Chloropyrifos	(1.4)	
	(0.01)	
<i>B.c/ NOECs-43KDa</i>		
<i>Microtox-EC50s</i>	<i>B.c/ NOECs-67KDa</i>	
:	<i>B.c/ NOECs-67KDa</i>	<i>B.c/ NOECs-43KDa</i>
		20000x
<i>B.c/ NOECs-43KDa</i>		
<i>1H EC50s Daphnia magna</i>	<i>B.c/NOECs-67KDa</i>	
	<i>B.c/ NOECs-43KDa</i>	
Chloropyrifos	(16.4)	
<i>B.c/ NOECs-67KDa</i>		
<i>1H EC50s Daphnia magna</i>		
(15.6)	(2.5)	
(1) Chloropyrifos		(8.2)
<i>B.c/ NOECs-67KDa</i>	<i>B.c/ NOECs-43KDa</i>	

	NOECs-43KDa	.1
() Reproductive-NOECs	
NOECs-67KDa	Cypermethrin	
	Reproductive-NOECs	
	NOECs-43KDa	.2
Dimethylphenol	NOECs-67KDa	
	Chloropyrifos	
<i>B.c/</i> NOECs-67KDa	<i>B.c/</i> NOECs-43KDa	.3
)		
	(
.LC50s	Reproductive-NOECs	.4
		•
	(67KDa 43KDa)	
		•
		•

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