Zinc acetate lozenges for the treatment of the common cold: a randomized controlled trial

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Table S1: Potential explanations for the negative results in 12 trials on zinc lozenges

Trial [Ref.]	**Salt and relevant ingredients**	**Zn dose (mg/day)**	**Problems in the lozenge composition and other problems**
Turner 2000 [9] Induced colds	Zinc acetate	69	"… hydrogenated palm kernel and cotton seed oils were also constituents of the lozenges, according to the list of ingredients provided with the commercial product (Halls Zinc Defense) marketed by Warner Lambert, which is also the supplier of the zinc acetate lozenge clinical prototypes studied by Turner et al. [1]. At the high temperatures (157°C) used in the manufacture of hard candy, these ingredients react with positively charged zinc ions (Zn^{2+} ions) derived from zinc acetate to yield zinc oleate, stearate, and palmitate waxes, which are incapable of releasing Zn^{2+} ions." **Ref 17** https://doi.org/10.1086/320177 Turner did not respond to this criticism, which indicates that the criticism has not been refuted. + low dose **Ref 18 and 19** Zinc dose shown on the left-hand side is the planned dose, but the actual dose used by participants was not reported.
Turner 2000 [9] Natural colds	Zinc acetate	69	The same
Turner 2000 [9] Induced colds	Zinc acetate	30	The same
Turner 2000 [9] Natural colds	Zinc acetate	30	The same
Farr 1987 [10] Trial 1	Zinc + citrate	184	"Farr et al. … , 90 mg citric acid (2% of lozenge weight) … The significance of the added citric acid was unknown until a 1988 article by solution chemist R. Bruce Martin, Ph.D., was published showing the absence of ionic zinc and presence of negatively charged zinc species at physiologic pH [27]. …. The reaction product was tightly bound zinc citrate" (p 484) **Ref 16** https://doi.org/10.1016/j.mehy.2009.10.017 See also about zinc and citrate: DOI: 10.1128/AAC.32.4.605 DOI: 10.1128/AAC.32.4.606 DOI: 10.1128/AAC.32.4.608 DOI: 10.1002/jps.2600810205 DOI: 10.3184/095422999782775672 "lozenge … contained 23 mg of elemental zinc… A total of eight doses was administered each day" (p 1183-4) which sums to 184 mg/day of elemental zinc.
Farr 1987 [10] Trial 2	Zinc + citrate	184	The same

Douglas 1987 [11]	Zinc + tartrate + carbonate	64	"The Douglas et al. [22] 1987 RCT report omitted mention of additive food acids in their ‘‘effervescent” zinc acetate lozenges… A letter from the lozenge designer and manufacturer, Faulding LTD, Adelaide, South Australia, indicated that the lozenges contained zinc acetate plus tartaric acid and sodium bicarbonate sufficient to result in strong oral effervescence [16]. Zinc acetate dissociates in the presence of these added ingredients and forms several tightly bound reaction products including zinc carbonate, which is non-soluble and non-ionizable [38] and negatively charged zinc tartrate species [39]." **Ref 16** https://doi.org/10.1016/j.mehy.2009.10.017 + low dose, see **Refs 18 and 19**
Smith 1989 [12]	Zinc gluconate + mannitol + sorbitol	207	"The lozenge of the Smith et al. trial contained mannitol and sorbitol. There is experimental evidence that mannitol and sorbitol bind zinc ions in the presence of saliva, which may explain the negative findings in the Smith et al. trial. Furthermore, Dr Smith was one of the authors of the Godfrey et al. trial, which stated in its introduction (p.235) that ‘it has been demonstrated that . . . mannitol/sorbitol inactivate zinc by chelation in saliva’ and ‘mannitol/sorbitol [zinc lozenge] formulations release no zinc ions when dissolved in the mouth’ referring to the Smith et al. trial. This indicates that afterwards Dr Smith did not trust the lozenge formulation of his 1989 trial." **Ref 19** https://doi.org/10.1177%2F2054270417694291 See also introduction in the Godfrey (1992) paper: DOI: 10.1177/030006059202000305
Weismann 1990 [13]	Zinc gluconate	45	Low dose, see **Refs 18 and 19**
Macknin 1998 [14]	Zinc gluconate	45	Low dose, see **Refs 18 and 19**
Eby 2006 [15]	Zinc orotate	273	"Zinc orotate is tightly bound (0 mg iZn) and essentially insoluble [50], and non-soluble compounds do not release iZn… Lozenges were nearly insoluble and required more than 1 h to dissolve in the mouth. This study was the second component of our 1984 clinical trial [21], and its results were published in 2 mid-90s books [16,17], but were not published as a peer reviewed article until 2006." (p 485) **Ref 16** https://doi.org/10.1016/j.mehy.2009.10.017 "lozenges containing either 37 mg zinc… One lozenge was dissolved in the mouth every 2 to 3 wakeful hours" (p 1183-4) which sums to 273 mg/day of elemental zinc, assuming 16 h awake and 2.5 hour interval.

Turner 2000 [9]	Zinc gluconate **Natural colds** A parallel Turner (2000) trial with the same zinc gluconate lozenge found significant increase (P = 0.035) in the recovery rate from **induced rhinovirus colds.** See p 9-11 of this supplement.	80	This may be the only one of the 12 negative trials that does not have a clearly plausible explanation for the lack of benefit from the zinc lozenges, but there are possible explanations. First, the calculation of dose 80 mg/day is based on the planned frequency of lozenge usage and not on reported/observed frequency of usage. In the current trial by Hemilä (2020) the ratio of actual usage to planned usage was 5.1/6.0 = 85%. If the same ratio applied to Turner (2000) trial, the actual dose would have been 68 mg/day (= 0.85*80). Mossad (1996)[5], Petrus (1998)[6], Prasad (2000)[7] and Prasad (2008)[8] asked about the actual use of lozenges and therefore their dose estimates 80-92 mg/day are based on the reported usages and not on the planned usage. It does not seem likely that a difference between 68 mg/day and 80-92 mg/day could render the Turner zinc gluconate lozenge ineffective, but somewhat low dose may be part of the explanation for their negative finding. Second, Mossad (1996)[5], Prasad (2000)[7] and Prasad (2008)[8] required that colds had lasted <24 hours and nearly all of the participants in Petrus (1998)[6] had colds <24 hours. Turner (2000)[9] included participants who had colds <36 hours and this longer delay between the start of symptoms and the start of treatment is also a potential reason for low efficacy in the Turner natural colds trial, assuming that rapid initiation of treatment might be optimal. Finally, the same lozenge was effective (P = 0.035) in a parallel trial with induced rhinovirus type 39 colds. It is possible that the effect of zinc lozenges varies between viruses so that the discrepancy between the findings for natural colds and induced colds might be partly explained by the types of viruses causing the symptoms.

Statistical calculations for the Hemilä (2020) trial

All participants (n = 87)

```
> CrossTable(Helzinki$Duration,Helzinki$Zinc, prop.r ="F", prop.c ="F", pr
op.t ="F", prop.chisq ="F")


Total Observations in Table:  87


                   | Helzinki$Zinc
Helzinki$Duration |         0 |         1 | Row Total |
------------------|-----------|-----------|-----------|
                2 |         4 |         5 |         9 |
------------------|-----------|-----------|-----------|
                3 |         6 |         4 |        10 |
------------------|-----------|-----------|-----------|
                4 |         5 |         5 |        10 |
------------------|-----------|-----------|-----------|
                5 |         9 |         4 |        13 |
------------------|-----------|-----------|-----------|
                6 |         7 |         1 |         8 |
------------------|-----------|-----------|-----------|
                7 |         0 |         9 |         9 |
------------------|-----------|-----------|-----------|
                8 |         1 |         3 |         4 |
------------------|-----------|-----------|-----------|
                9 |         2 |         2 |         4 |
------------------|-----------|-----------|-----------|
               10 |         8 |        12 |        20 |
------------------|-----------|-----------|-----------|
     Column Total |        42 |        45 |        87 |
------------------|-----------|-----------|-----------|


> CrossTable(Helzinki$Cured,Helzinki$Zinc, prop.r ="F", prop.c ="F", prop.
t ="F", prop.chisq ="F")


Total Observations in Table:  87


               | Helzinki$Zinc
Helzinki$Cured |         0 |         1 | Row Total |
---------------|-----------|-----------|-----------|
             0 |         5 |        11 |        16 |
---------------|-----------|-----------|-----------|
             1 |        37 |        34 |        71 |
---------------|-----------|-----------|-----------|
  Column Total |        42 |        45 |        87 |
---------------|-----------|-----------|-----------|
```

All participants (n = 87)

```
> RR <- coxph(zincsurv ~ HelZinki$Zinc, method = "efron")
> RR
Call:
coxph(formula = zincsurv ~ HelZinki$Zinc, method = "efron")

                 coef exp(coef) se(coef)     z   p
HelZinki$Zinc -0.393     0.675    0.239 -1.64 0.1

Likelihood ratio test=2.7  on 1 df, p=0.1
n= 87, number of events= 71

> exp(confint(RR))
                2.5 % 97.5 %
HelZinki$Zinc 0.4223  1.079
```

No sinusitis subgroup (n = 59)

```
> NoSinusitis <- subset(HelZinki, Sinusitis==0)
> survNoSinus <- Surv(NoSinusitis$Duration, NoSinusitis$Cured)
>
> RR <- coxph(survNoSinus ~ NoSinusitis$Zinc, method = "efron")
> RR
Call:
coxph(formula = survNoSinus ~ NoSinusitis$Zinc, method = "efron")

                   coef exp(coef) se(coef)     z    p
NoSinusitis$Zinc -0.428     0.652    0.291 -1.47 0.14

Likelihood ratio test=2.1  on 1 df, p=0.147
n= 59, number of events= 49
> exp(confint(RR))
                  2.5 % 97.5 %
NoSinusitis$Zinc 0.3685  1.153
```

Participants with NO side effects (n = 56)

"tas_oth" variable indicates taste or other side effects (SE)

```
> tas_oth <- subset(HelZinki, tas_othSE==0)
> tas_oth$tas_othSE
 [1] 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
> tas_oth$tas_othANY
NULL
>
> tosurv <- Surv(tas_oth$Duration, tas_oth$Cured)
> toRR <- coxph(tosurv ~ tas_oth$Zinc, method = "efron")
> toRR
Call:
coxph(formula = tosurv ~ tas_oth$Zinc, method = "efron")

               coef exp(coef) se(coef)     z    p
tas_oth$Zinc -0.179     0.836    0.313 -0.57 0.57

Likelihood ratio test=0.33  on 1 df, p=0.565
n= 56, number of events= 48

> exp(confint(toRR))
               2.5 % 97.5 %
tas_oth$Zinc 0.45258 1.5458
```

Participants WITH side effects (n = 31)

```
> ytas_oth <- subset(HelZinki, tas_othSE>0)
> ytas_oth$tas_othSE
 [1] 6 3 6 3 6 3 6 2 4 2 3 2 1 4 6 6 5 4 4 1 3 1 4 3 1 6 1 2 5 3 3
>
>
> ytosurv <- Surv(ytas_oth$Duration, ytas_oth$Cured)
> yRR <- coxph(ytosurv ~ ytas_oth$Zinc, method = "efron")
> yRR
Call:
coxph(formula = ytosurv ~ ytas_oth$Zinc, method = "efron")

                coef exp(coef) se(coef)     z     p
ytas_oth$Zinc -1.138     0.320    0.584 -1.95 0.051

Likelihood ratio test=3.1  on 1 df, p=0.0782
n= 31, number of events= 23
> exp(confint(yRR))
                2.5 % 97.5 %
ytas_oth$Zinc 0.10195 1.0065
```


Hemilä H, *et al. BMJ Open* 2020; 10:e031662. doi: 10.1136/bmjopen-2019-031662

Participants still sick on the 4th day and cured by the 7th day

```
> Day7 = matrix(c(11,21,26,10), nrow = 2)
> Day7
     [,1] [,2]
[1,]   11   26
[2,]   21   10
>
>
> fisher.test(Day7)

        Fisher's Exact Test for Count Data

data:  Day7
p-value = 0.0031
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
 0.062983 0.631049
sample estimates:
odds ratio
   0.20682
```

Table S2: **Analysis Turner (2000) zinc gluconate trial with induced rhinovirus colds**
https://doi.org/10.1086/317437
Extraction of recovery data from Figure 1A for Placebo and Zn gluconate
The published figure was measured as pixel units and transformed to patients:

Turner 2000 experimental colds; Figure 1A																		
	Zn gluconate (n=69)									Placebo (n=67)								
Day	pixels	difference pixels	To Fig scale (0 to 100%)	To persons 69	Difference	To person integers	Cured per period		Day	pixels 100%= 2714	difference pixels	To Fig scale (0 to 100%)	To persons 67	Difference	To person integers	Cured per period	
0	139		100,0	69,0		69	0	0	0	139		100,0	67,0		67	0	0
0,5	513		85,5	59,0	10,0	59	10	10	0,5	371		91,0	61,0	6,0	61	6	6
1	658		79,8	55,1	3,9	55	4	14	1	482		86,7	58,1	2,9	58	3	9
1,5	1071		63,8	44,0	11,1	44	11	25	1,5	713		77,7	52,1	6,0	52	6	15
2	1182		59,5	41,1	3,0	41	3	28	2	830		73,2	49,0	3,0	49	3	18
2,5	1558		44,9	31,0	10,1	31	10	38	2,5	1139		61,2	41,0	8,0	41	8	26
3	1782		36,2	25,0	6,0	25	6	44	3	1330		53,7	36,0	5,0	36	5	31
3,5	2048		25,9	17,8	7,1	18	7	51	3,5	1676		40,3	27,0	9,0	27	9	40
4	2162	114	21,4	14,8	3,1	15	3	54	4	1981		28,5	19,1	7,9	19	8	48
4,5	2200	38	20,0	13,8	1,0	14	1	55	4,5	2104	123	23,7	15,9	3,2	16	3	51
5	2234	34	18,6	12,9	0,9	13	1	56	5	2104		23,7	15,9	0,0	16	0	51
5,5	2311	77	15,7	10,8	2,1	11	2	58	5,5	2141	37	22,3	14,9	1,0	15	1	52
6	2352	41	14,1	9,7	1,1	10	1	59	6	2141		22,3	14,9	0,0	15	0	52
6,5	2352		14,1	9,7	0,0	10	0	59	6,5	2141		22,3	14,9	0,0	15	0	52
7	2390	38	12,6	8,7	1,0	9	1	60	7	2182	41	20,7	13,8	1,1	14	1	53
7,5	2390		12,6	8,7	0,0	9	0	60	7,5	2218	36	19,3	12,9	0,9	13	1	54
8	2427	37	11,1	7,7	1,0	8	1	61	8	2303	85	16,0	10,7	2,2	11	2	56
8,5	2500	73	8,3	5,7	2,0	6	2	63	8,5	2345	42	14,3	9,6	1,1	9	2	58
9	2500		8,3	5,7	0,0	6	0	63	9	2388	43	12,7	8,5	1,1	8	1	59
9,5	2500		8,3	5,7	0,0	6	0	63	9,5	2430	42	11,0	7,4	1,1	7	1	60
10	2572	72	5,5	3,8	1,9	4	2	65	10	2430		11,0	7,4	0,0	7	0	60
10,5	2607	35	4,2	2,9	0,9	3	1	66	10,5	2430		11,0	7,4	0,0	7	0	60
11	2684	77	1,2	0,8	2,1	1	2	68	11	2470	40	9,5	6,3	1,0	6	1	61
11,5	2717	33	-0,1	-0,1	0,9	0	1	69	11,5	2470		9,5	6,3	0,0	6	0	61
12								69	12	2470		9,5	6,3	0,0	6	0	61
12,5									12,5	2510	40	7,9	5,3	1,0	5	1	62
13									13	2510		7,9	5,3	0,0	5	0	62
13,5									13,5	2510		7,9	5,3	0,0	5	0	Censored
										Censor	204						
						n =	69								n =	62	

Zn gluconate (n=69)			Placebo (n=67)		
Day	Cured per period		Day	Cured per period	Censored
0.5	10		0.5	6	
1.0	4		1.0	3	
1.5	11		1.5	6	
2.0	3		2.0	3	
2.5	10		2.5	8	
3.0	6		3.0	5	
3.5	7		3.5	9	
4.0	3		4.0	8	
4.5	1		4.5	3	
5.0	1		5.0	0	
5.5	2		5.5	1	
6.0	1		6.0	0	
6.5	0		6.5	0	
7.0	1		7.0	1	
7.5	0		7.5	1	
8.0	1		8.0	2	
8.5	2		8.5	2	
9.0	0		9.0	1	
9.5	0		9.5	1	
10	2		10	0	
10.5	1		10.5	0	
11	2		11	1	
11.5	1		11.5	0	
			12	0	
			12.5	1	
			13	0	
			13.5		5
Total	**69**			**62**	**5**

```
> CrossTable(Turner$days,Turner$zinc, prop.r ="F", prop.c ="F", prop.t ="F
", prop.chisq ="F")


Total Observations in Table:  136
```

Turner$days	Turner$zinc 0	1	Row Total
0.5	6	10	16
1	3	4	7
1.5	6	11	17
2	3	3	6
2.5	8	10	18
3	5	6	11
3.5	9	7	16
4	8	3	11
4.5	3	1	4
5	0	1	1
5.5	1	2	3
6	0	1	1
7	1	1	2
7.5	1	0	1
8	2	1	3
8.5	2	2	4
9	1	0	1
9.5	1	0	1
10	0	2	2
10.5	0	1	1
11	1	2	3
11.5	0	1	1
12.5	1	0	1
13.5	5	0	5
Column Total	67	69	136

```
> survTurner <- Surv(Turner$days, Turner$cured)
> RR <- coxph(survTurner ~ Turner$zinc, method = "efron")
> summary(RR)
Call:
coxph(formula = survTurner ~ Turner$zinc, method = "efron")

  n= 136, number of events= 131

               coef exp(coef) se(coef)     z Pr(>|z|)
Turner$zinc 0.3740    1.4536   0.1768 2.115   0.0344 *
---
Signif. codes:
0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

            exp(coef) exp(-coef) lower .95 upper .95
Turner$zinc     1.454      0.688     1.028     2.056

Concordance= 0.554  (se = 0.029 )
Rsquare= 0.032   (max possible= 1 )
Likelihood ratio test= 4.48  on 1 df,   p=0.03419
Wald test            = 4.47  on 1 df,   p=0.0344
Score (logrank) test = 4.53  on 1 df,   p=0.03339

> exp(confint(RR))
                2.5 %   97.5 %
Turner$zinc 1.027852 2.055642
```

######################

The logrank P calculated above (P = 0.03339) is consistent with the logrank P reported by Turner (2000).
https://doi.org/10.1086/317437

Nevertheless, Turner did not publish the effect of zinc lozenges on the RR scale and therefore the calculation is done above.

Turner reported:

"Between-group comparisons of the time to cold resolution were performed by means of the log-rank test, adjusted for study site"
(p 1203, right-hand column)

"The median duration of illness in zinc gluconate recipients was 2.5 days, in comparison with 3.5 days in the placebo recipients (P = .035)."
(p 1204 left-hand column)