

# A true alternative to formaldehyde for egg disinfection

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The hatchery industry is a very international business which is very much in tune with new developments in the market worldwide. This is also reflected in the way information is shared by specialists communicating through scientific seminars, world congresses and specialist press.

Banning formaldehyde out of the hatchery for egg disinfection is definitely one of those hot topics today. It is a well known fact that legislation in terms of the exposure limit of formaldehyde to the people working in the hatchery is getting stricter by the day for many years now.

There are two ways of defining the exposure limits to formaldehyde. The first way gives a combination of a time-weighted-average concentration over eight hours (TWA) and a short-term-exposure-limit during 15 min (STEL), from which the values can vary from country to country. The TWA in the Netherlands for example is 0.12ppm, whereas in France it is 1ppm. The STEL has more or less the same variation.

The second way is the MEL, the maximum exposure limit. This is the strictest legislation which states that nobody can be exposed to more than 0.3ppm formaldehyde at all times. Belgium, Canada and Denmark for example have implemented this legislation.

It is fair to say that the exposure limits to formaldehyde and according legislations have become stricter every year, regardless which method of monitoring (TWA, STEL or MEL) is opposed.

Therefore, the difference between

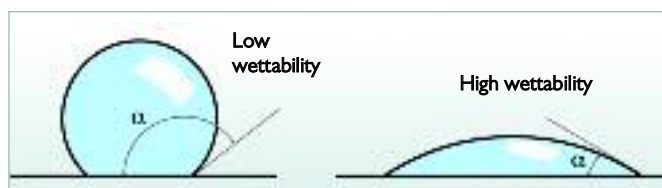


Fig. 1. The contact angle of small and large droplets.

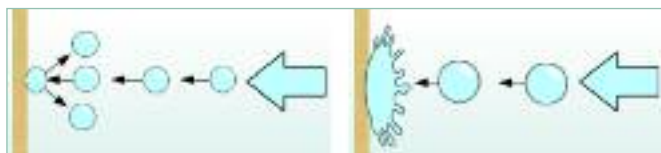


Fig. 2. With an ultrasonic fogger the droplets are so small they bounce against the surface without touching it and the eggs remain dry.

several years ago and today is that the hatcheries are not only talking about it, but are also taking action.

Tests are set up everywhere with various products and applications in order to find a true alternative for formaldehyde.

## Standing still is moving back

It is clear that these human health issues are putting a lot of pressure on the current formaldehyde protocols implemented in the hatcheries.

In 1953 Lancaster & Crabb found that, in order to kill *S. pullorum* on the eggshell using a 20 minutes fumigation period, a minimum concentration of 600mg formaldehyde per m<sup>3</sup> (10g paraformaldehyde or 45ml 40% formalin and 30g KMnO<sub>4</sub>) at 21 °C is necessary.

However, this protocol has proven a certain degree of efficacy on eggs that were contaminated  $\leq \log_4$  (eggs with  $\geq \log_5$  contamination, complete disinfection of the shell surface by fumigating formaldehyde at 10g/m<sup>3</sup> is not possible), it is

quite remarkable that still today, well over 50 years later, most of the hatcheries are still following this same protocol!

It is needless to say that the human health issue in 1953 was not taken into account or was less relevant. So the question lingers: how well does this 'old' formaldehyde protocol relate to the very low exposure limits of today's legislation?

## The only way is out!

The reason why this amount of 10g paraformaldehyde per m<sup>3</sup> was never exceeded, is because research has shown a significant relationship between embryonic mortality, duration of fumigation and the concentration of formaldehyde.

A significant decrease (8%) in hatchability was reported when the formaldehyde fumigations were used at higher duration (40 minutes) and higher concentration (12.5g/m<sup>3</sup>).

So, from an economical point of view, using more formaldehyde was never an option. Now with a MEL of

0.3ppm it is definitely out of the question. In fact, a lot of hatcheries are already struggling to maintain the same levels of formaldehyde (10g/m<sup>3</sup>) and at the same time be in compliance with legislation.

Basically the problem lies with infrastructure difficulties. If the same amount of formaldehyde is used it will mean that bigger air evacuation systems must be implemented and a much longer air evacuation time needs to take place before people can enter the fumigation rooms. In practice this turns out to be an almost impossible nut to crack.

Two hours after air evacuation and fumigation of only 5g/m<sup>3</sup> of paraformaldehyde, the STEL of 0.3ppm was still exceeded by 14 times! Therefore, using less formaldehyde does not really make it any easier to stay working within the allowed exposure limits. It also of course raises questions on its bactericidal efficacy.

## A true alternative

On 11th October 2011 CID LINES was invited to the first CEVA hatchery university in Madrid to give a lecture on VIROCID as an alternative for formaldehyde for the disinfection of hatching eggs before setting.

More than 40 supervising veterinarians, plant managers and quality supervisors from the entire Spanish hatchery industry attended this seminar.

The presentation was based on an extensive trial where beside the efficacy of VIROCID (combination of glutaraldehyde and multi chain quaternary ammonium) the safety aspect for human health and the hatchability were also included as

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Table 1. Summary of methods and results of several trials.

	Dilution (%)	Consumption	Fogging time	Circulation during fumigation	Circulation time after fogging	Ventilation time (air extraction)	Log reduction
Formaldehyde (paraformaldehyde)		450g	20	YES	NO	40	2.11 <sup>a</sup>
VIROCID ultrasonic fogging	20	420ml	20	NO	10 min	30	2.21 <sup>a</sup>
VIROCID cold fogging 1	10	220ml	20	NO	25 min	15	2.86 <sup>b</sup>
VIROCID cold fogging 2	20	420ml	20	NO	25 min	15	3.12 <sup>b</sup>

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parameters. VIROCID was applied by ultrasonic fogging and cold fogging. The major difference between these two fogging principles is the droplet size of the fog that is generated. Ultrasonic foggers output between 1-5µm and the cold fogger that was used gave droplet sizes ranging from 20-25µm.

### Trial set-up

The trials took place in a commercial hatchery with full disinfection rooms. The room held about 30 trolleys. For each of those testing groups, 60 eggs were swabbed, each time out of five trolleys that were always located at the same place in the fumigation room.

In addition, the trays from which the eggs were swabbed were always the same: middle tray, three trays up and three trays down. Using fixed locations will not only tell something about the dispersing of the fog and its disinfection power, but also minimises unwanted variations between the trials.



**The swab method.**

The temperature in the fumigation room was always the same between trials as well as the temperature of the eggs at the start of fumigation.

The eggs were swabbed with 'wet-cotton-swab method' (COPAN, rinse kit). In this way 95% of the egg shell surface could be swabbed which gives a much higher, but also a much more accurate, bacteria count than would have been the case if eggs were sampled by contact agar plates.

For every egg new gloves were used to avoid contamination between eggs by the fingers of the swab taker.

Every time the two batches, one that was fumigated with formaldehyde and the other fogged with VIROCID, were swabbed. The eggs compared within the batches were originated from the same flock/house with the eggs having a similar age.

After fogging and air evacuation times, the air was sampled in the

Group	Trial group	Control group
Animal type	Layers	Layers
Hatching eggs	Broilers	Broilers
No. of flocks (origin)	7	7
No. of houses	13	13
Age range	32-48	31-45
Total amount of eggs	863,100	1,389,150
Candling (%)	12.33	12.18
Hatchability (%)	81.73	81.17

**Table 2. Comparison of eggs disinfected with VIROCID and those disinfected with formaldehyde.**

fumigation room to measure the remaining glutaraldehyde in the air.

For glutaraldehyde (VIROCID) the maximum exposure limit is 0.05ppm. Thus, in order to be in compliance with legislation people cannot enter the fumigation room before the MEL is below 0.05ppm.

### Disinfection

Several trials were done repetitively. We have chosen to keep the complete procedure limited to one hour, from start of fogging to taking out the trolleys for setting (personnel entry).

In Table 1 the methods and results are summarised. There is a significant difference between the log reduction of formaldehyde and VIROCID. In ultrasonic fogging VIROCID has the same disinfection value as formaldehyde. In cold foggers VIROCID is even significantly better than formaldehyde. This can be explained by the different droplet sizes of both fogging principles. A very small droplet has a big contact angle, a bigger droplet a smaller contact angle (see Fig. 1).

The contact angle will determine the wettability. The wetter a surface gets by a disinfectant solution, the more the solution can act upon that surface, and therefore disinfect. That is also why the eggs when fogged with an ultrasonic fogger stay dry. The droplets are so small they bounce against the surface without touching it (see Fig. 2). With cold fogging the eggs are slightly moist.

### Creating an ideal protocol

Furthermore, we can learn from these trials that a double disinfection could be the ideal protocol for well disinfected eggs and unharmed embryos.

Given the fact that the actual log reduction with formaldehyde is relatively low and even with a double concentration – which is for today's

strict exposure limits absolutely not feasible – eggs with a bacterial contamination  $\geq \log 5$  are impossible to disinfect completely, we should start the first disinfection on farm level.

After separation from the hen at oviposition, the egg is constantly exposed to contaminations. It is crucial to destroy micro-organisms while they are still on the egg shell.



**Sampling the air in the fumigation room.**

Once micro-organisms penetrate the shell, they reach the shell membrane within minutes and are protected from the disinfectant.

Ideally, the first disinfection should take place on farm level as soon as possible, preferably when eggs are still warm. The second best option is to disinfect during transport. Afterwards the second disinfection can take place in the hatchery.

Trying to disinfect properly (log4-log5) in only one phase is asking for trouble.

### Hatchability

The trial batches were also followed up to hatching, where candling and hatchability was analysed.

The ultimate goal is to obtain a high hatchability percentage and quality chicks. The eggs disinfected

with VIROCID were compared to those disinfected with formaldehyde. The results are shown in Table 2. There is no significant difference between the trial group and the control group, thus no negative effect on hatchability can be noted in this trial when eggs are disinfected with VIROCID compared to formaldehyde.

### Maximum exposure limit

The determination of MEL was done by air sampling. The aim is to define if personnel entry after 30 minutes active ventilation is possible and in compliance with legislation.

With VIROCID we stay under the 0.05ppm exposure limit and the disinfecting procedure from start to finish can be concluded within the hour.



In conclusion, VIROCID is a true alternative for formaldehyde for egg disinfection, where log reductions are equal or significantly better than formaldehyde, hatchability is not influenced negatively and is in compliance with MEL legislation. ■

*References are available from the author on request*

**Table 3.**

Measured volume: 3,004 L	Result ppm	Confidence interval ppm	TLV ppm	MEL ppm	Notation	LOD ppm	LOQ ppm	CV <sub>an</sub> %	CV <sub>tot</sub> %
Glutaraldehyde	0.049	±0.006	-	0.05	M	0.003	0.013	4.0	6.4