SUPPLEMENTAL INFORMATION FOR OAE

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One benefit of oxalic acid is its rapid degradation, which is also a problem for efficacy when colonies contain brood. The Api-Bioxal label states "Use Oxalic Acid Dihydrate when little or no brood is present." That describes the problem. If applied by dribble, OA is not efficacious *as a single treatment* in colonies containing brood, plus repeated dribbles cause adverse effects on the colony, not to mention the aforementioned risk involved in removing and restacking the honey supers. Oxalic acid applied by vaporization in hot weather requires multiple applications for efficacy.

THE RAPID DEGRADATION AND RESIDUAL ACTIVITY OF OXALIC ACID

EPA is aware that oxalic acid rapidly degrades in the environment, and the FDA is not concerned as far as contamination of the hive, comb, or honey. The mode of action of oxalic acid appears to be solely due to its acidity, rather than the oxalate ion [ⁱ], thus its residual activity can be quantified via acid-base titration. Figure 1 shows my own data from a bench trial in which various applications of oxalic acid were applied to freeze-killed bees held at room temperature, as well as control drops of solution placed on plastic.



Fig. 1. Rates of degradation of acidity of oxalic acid in solutions or vapor applied to cover slips or dead, dry bee bodies, at room temperature or frozen [ⁱⁱ]. Oxalic acid is stable, once dried on plastic, but rapidly degrades if is exposed to bee chitin, sugar, or glycerin.

Due to its rapid degradation, oxalic acid, applied to honey bees in the hive, exhibits minimal risk to man or the environment. That said, it also means that due to its limited residual activity, oxalic acid — applied by the approved methods — exhibits poor efficacy when colonies contain brood. Thus, the Aluen CAP project in Argentina came up with an extended-release method, which although currently widely used throughout the world (and the United States) is yet to be approved by EPA.

EXPLAINING THE EXTENDED-RELEASE METHOD FOR OXALIC ACID

The extended-release method involves dissolving the OA in glycerin (as a humectant), allowing the solution to soak into cellulose sponges or chipboard strips, and then inserting the sponges or strips into the hive, where adult bees come in contact with the acid, which then sticks to their exoskeletons. This method, even at applied doses of up to 50 grams of OA in the matrix (much of which is never distributed to the bees), exhibits no adverse effects upon the adult bees or brood, and *application is less risky for the beekeeper* than any of the currently-approved application methods for Api-Bioxal.

CONCERNS ABOUT THE PREPARATION

For the Argentinian ratio of 1 g of OA to 2 mL of glycerin, **no heating is necessary** – it is only when higher OA:gly ratios are desired that heating is required to dissolve the acid.

After having personally prepared a great number of batches of OAE sponges and strips, I find it to be no more hazardous than the safety concerns involved in handling a boiling a pot of hot water, or a far more hazardous pot of cooking oil at 350°F (either of which can cause severe and immediate burns), or using off-the-shelf wood bleach, muriatic acid, oven cleaners, or drain opener (all concentrated strong acids or bases). Oxalic acid is not that much stronger than the citric acid commonly used in cooking.

So long as safety glasses are worn, there is little risk involved. A spill of the OA-glycerin solution on the skin causes no immediate noticeable damage, and is easily rinsed off without need for panic, and any acid residues in the preparation area, equipment, or clothing are easily neutralized with a solution of baking soda. Kits to prepare OAE strips are legally sold in New Zealand without incident.

CONCERNS ABOUT THE DOSAGE

EPA ruled against the states that passed 2ee exemptions for OAE, due to concerns about the increased dosage, but the question is, why is EPA concerned about the dosage?

Although the currently-approved Directions for Use of oxalic acid allow for application of only 1.0 grams of the dihydrate per brood chamber *per application by vaporization*, the label does not restrict the interval period between applications nor limit the total number of applications that can be given. *Thus, there is no legal restriction upon the number of applications, nor the total dosage of OA that can be applied to a colony per day*. Therefore, a beekeeper is legally free to apply as large a dose of Api-Bioxal to a hive as they choose.

The above said, despite that (presumably unintentional) oversight, the EPA may have a legitimate concern about increasing the dose of OA applied to *a hive* (the cavity in which the honey bee colony nests). But shouldn't EPA instead focus upon the dose of OA *per individual bee?*

THE CALCULATED OA DOSAGE PER BEE

| Calculated approved per/bee | dosages d | of oxalic a | acid per | bee |
|-----------------------------|-----------|-------------|----------|-----|
| | | | | |

| Solution Method | | |
|-----------------|----------------------|--|
| 35 | g OA | |
| 1000 | mL sugar syrup | |
| 35000 | μg OA/mL | |
| 5 | mL applied seam | |
| 175000 | μg OA/seam of bees | |
| 2000 | bees/seam | |
| 87.5 | μg/bee approved dose | |

| Spraying Package Bees Method | | |
|------------------------------|--------------------------|--|
| Solution the same | | |
| 3 | mL applied per 1000 bees | |
| 105 | μg/bee approved dose | |
| | | |

| Vaporizer Method | | |
|------------------|-------------------------------------------------------------------------------------------------|--|
| 1 | g OA/brood chamber | |
| 20,000 | bees per brood chamber | |
| 50 | μg/bee by label | |
| 50 | μg /bee for an efficacious dose for colonies not containing brood | |
| 150 | μg/bee for an efficacious dose of 3 g OA per brood chamber for colonies with brood | |

The above calculations indicate that all three approved application methods theoretically apply an average dose of 50-105 micrograms of oxalic acid per bee, although the efficacious dose (via vaporization) for colonies with brood is higher, based upon practical experience and recent research [ⁱⁱⁱ].

ACTUAL DOSE RECEIVED BY EACH BEE, DETERMINED BY TITRATION

My own data via titration indicates that *there's a big difference between the "dose" applied "to the hive," and the actual dose that shows up on the bees*. My titration data of adult bees immediately taken from the combs after various application methods indicates that there is great bee-to-bee variation in the received dose (not shown), and that the applied dose by the approved methods rapidly degrades.

On the other hand, an extended-release application of up to even 50 grams of OA, dissolved in glycerin, and applied in cellulose matrices, initially results in, and then maintains, a lower *per bee* dose of OA (both immediate and residual) no higher than the already-approved application methods (which provide ineffective varroa control when colonies contain brood) (Figure 2).



Fig 2. Oxalic acid rapidly degrades once applied to bees' bodies (alive or dead), or when mixed with glycerin or sugar [^{iv}], and after any application method, quickly drops to low levels of acidity. The theoretical per-bee dose of 100 μ g is seldom attained by any application method, and the mean per-bee residual acidity by any method rapidly drops to less than 10 μ g. I have additional more-detailed data in prep.

The 50-gram dose contained in a sponge is an artifact of the amount of solution that the matrix will absorb, rather than the delivered dose (efficacy is dependent upon the surface area of the delivery matrix, rather than the amount of OA absorbed). Published findings [^{v, vi}] indicate that high efficacy can be obtained with total applied doses in the 20-40 gram range, much of which is removed by the bees and scattered on the ground to quickly degrade.

Take home point: As far as *exposure of the bees to the active ingredient*, application of oxalic acid by the extended-release method is little different than applying it by currently-approved repeated applications, and actually results in lower acidity on the bees than with repeated applications.

ⁱ <u>http://varroa.fr/wp-content/uploads/2017/08/Fries-I.-et-al-1999.-Coordination-in-europe-of-integrated-control-of-varroa-mites-in-honey-bees-colonies.pdf</u>

[&]quot; Oliver, Randy, in prep.

ⁱⁱⁱ Jack, C. J., van Santen, E., & Ellis, J. D. (2020) op cit.

^{iv} Oliver, Randy, in prep.

^v <u>https://scientificbeekeeping.com/extended-release-oxalic-acid-progress-report-2/</u>

^{vi} Kanelis, D., Tananaki, C., Liolios, V., & Rodopoulou, M. A. (2023). Evaluation of oxalic acid with glycerin efficacy against *Varroa destructor* (Varroidae): a four year assay. *Journal of Apicultural Research*, 1-9.