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Addendum: Quantitative Analysis of Honey Bee Blood-Ethanol Levels Following Exposure to Ethanol Vapors

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This addendum reports an additional statistical analysis of the data of our earlier paper on the effect of exposing bees to ethanol vapor. The analysis indicated that inhaled ethanol is absorbed into the hemolymph, similar to the more traditional method of feeding bees ethanol. Therefore, both ingestion and inhalation can be used as effective methods of ethanol administration in honey bees.

Keywords: addiction, ethanol, honey bee, inebriator, ethanol vapor, ethanol inhalation

補遺：エタノール蒸気に曝露したミツバチの血中エタノール濃度の定量分析

この補遺では、ハチをエタノール蒸気に曝露した場合の影響に関する先行論文のデータを追加統計分析した。分析によると、ハチにエタノールを摂取させる従来の方法と同じように、吸入されたエタノールは血リンパに吸収されることが示された。したがって、ミツバチへのエタノール投与は、摂取と吸入の両方を効果的な方法として用いることができ。

*キーワード：*依存症、エタノール、ミツバチ、inebriator、エタノール蒸気、エタノール吸入

Apéndice: Análisis Cuantitativo de los Niveles de Etanol en Sangre de Abejas Melíferas Tras la Exposición a Vapores de Etanol

Este apéndice informa sobre un análisis estadístico adicional de los datos de nuestro artículo anterior sobre el efecto de la exposición de las abejas al vapor de etanol. El análisis indicó que el etanol inhalado se absorbe en la hemolinfa, de forma similar al método más tradicional de alimentar a las abejas con etanol. Por lo tanto, tanto la ingestión como la inhalación pueden utilizarse como métodos eficaces de administración de etanol a las abejas melíferas.

Palabras clave: adicción, etanol, abeja melífera, inebriator, vapor de etanol, inhalación de etanol

Through the present Addendum, we want to add a statistical analysis for the data shown in Figure 2 of our recently published study “Quantitative Analysis of Honey Bee Blood-Ethanol Levels Following Exposure to Ethanol Vapors” (Stauch et al., 2025). Honey bees (*Apis mellifera*) were exposed to 95% ethanol vapors for 0, 1, 2.5, 5 or 7.5 min and, at 1 min post-exposure, hemolymph was collected for quantification of ethanol levels through gas chromatography. We add an ANOVA to evaluate the effect of exposure to ethanol vapors on the level of ethanol in the hemolymph of the bees.

Mean ethanol hemolymph levels increased with increasing durations of exposure to ethanol vapors (0 min: 0.868 mM; 1 min: 7.212 mM; 2.5 min: 28.469 mM; 5 min: 43.047 mM; 7.5 min: 82.340 mM). One-way ANOVA found a significant main effect of ethanol inhalation on hemolymph ethanol level ($F(4,61) = 14.825$, $p < .0001$). Post hoc analysis through Tukey's HSD test revealed that, compared to the control group (0 min of exposure to ethanol vapors), a significant increase in hemolymph ethanol was present starting from an ethanol exposure duration of 5 min (0 min vs 1 min: $p = .982$; 0 min vs 2.5 min: $p = .137$; 0 min vs 5 min: $p = .005$; 0 min vs 7.5 min: $p < .0001$). The 5 min exposure led to hemolymph ethanol levels that were significantly higher than for a 1 min exposure (5 min vs 1 min: $p = .025$). The 7.5 min exposure brought to hemolymph ethanol levels that were significantly higher than for any shorter exposure (7.5 min vs 1 min: $p < .0001$; 7.5 min vs 2.5 min: $p = .0002$; 7.5 min vs 5 min: $p = .011$).

These results indicate that inhaled ethanol is actually absorbed in the hemolymph of honey bees. Previous research has shown that ingested ethanol significantly raises ethanol level in the hemolymph (Božič et al., 2007). Hence, both ingestion and inhalation can be used as effective methods of ethanol administration in experimental studies investigating the effects of ethanol in honey bees.

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