**Inert agricultural spray adjuvants may increase the adverse effects of selected insecticides on honey bees (*Apis mellifera* L.) under laboratory conditions**

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# SUPPLEMENTARY INFORMATION (SI)

Fig S1: Hazard Ratio Full Trial A  
Tab S1: Mortality Data Full Trial A

Fig S2: Hazard Ratio Full Trial B  
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Tab S4: Mortality Data Full Trial D

Fig S5: Hazard Ratio Full Trial E  
Tab S5: Mortality Data Full Trial E

*Tab S1-S5: Mortality development during the test period*

The actual mortality of the contact toxicity test was corrected by the control mortality according to the SCHNEIDER-ORELLI formula (1947).

M = ((t –c)/(100 – c))x100 M = corrected mortality (%)  
t = mortality of the treatment group (%)  
c = mortality of the control group (%)

## **Supplementary Figure S1:** Hazard Ratio Full Trial A

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**Fig S1:** Hazard Ratios (HR) for two insecticide formulations and two spray adjuvants alone or their respective mixtures. All test substances were evaluated against the control within a 72-hour period. Bees from each of the two test colonies were divided into an equal number of cages (i.e. replicate) per treatment. The replicate could thus be evaluated as a covariate as well. (Cox proportional hazard model).

**Supplementary Table S1:** Mortality Data Full Trial A

**Tab S1:** Laboratory test 1 (November 2019): Mortality and corrected mortality M of the control and the eight treatments up to 72 h after application.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| *Treatment* | *cumulative mortality [%]* | | | | |  | *cumulative corrected mortality M [%]* | | | | |
| *+2* | *+4* | *+24* | *+48* | *+72* |  | *+2* | *+4* | *+24* | *+48* | *+72* |
| C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |  |  |  |  |  |  |
| 301 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Acx | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Mos | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Cor | 0.0 | 0.0 | 0.0 | 1.7 | 3.3 |  | 0.0 | 0.0 | 0.0 | 1.7 | 3.3 |
| Mos + 301 | 0.0 | 1.7 | 20.0 | 21.7 | 21.7 |  | 0.0 | 1.7 | 20.0 | 21.7 | 21.7 |
| Mos + Acx | 0.0 | 3.3 | 3.3 | 5.0 | 5.0 |  | 0.0 | 3.3 | 3.3 | 5.0 | 5.0 |
| Cor + 301 | 0.0 | 0.0 | 0.0 | 0.0 | 1.7 |  | 0.0 | 0.0 | 0.0 | 0.0 | 1.7 |
| Cor + Acx | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

Ncages/treatment=6; nbees/treatment=60

## **Supplementary Figure S2:** Hazard Ratio Full Trial B

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**Fig S2:** Hazard Ratios (HR) for three insecticide formulations and four spray adjuvants alone or their respective mixtures. All test substances were evaluated against the control within a 72-hour period. Bees from each of the two test colonies were divided into an equal number of cages (i.e. replicate) per treatment. The replicate could thus be evaluated as a covariate as well. (Cox proportional hazard model).

**Supplementary Table S2:** Mortality Data Full Trial B

**Tab S2:** Laboratory test 2 (December 2019): Mortality and corrected mortality M of the control and the 13 treatments up to 72 h after application.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| *Treatment* | *cumulative mortality [%]* | | | | |  | *cumulative corrected mortality M [%]* | | | | |
| *+2* | *+4* | *+24* | *+48* | *+72* |  | *+2* | *+4* | *+24* | *+48* | *+72* |
| C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |  |  |  |  |  |  |
| LI | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Has | 0.0 | 0.0 | 0.0 | 0.0 | 1.7 |  | 0.0 | 0.0 | 0.0 | 0.0 | 1.7 |
| Mos | 0.0 | 0.0 | 1.7 | 1.7 | 1.7 |  | 0.0 | 0.0 | 1.7 | 1.7 | 1.7 |
| Cor | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Kar | 0.0 | 5.0 | 76.7 | 76.7 | 76.7 |  | 0.0 | 5.0 | 76.7 | 76.7 | 76.7 |
| Mos + LI | 5.0 | 8.3 | 33.3 | 38.3 | 38.3 |  | 5.0 | 8.3 | 33.3 | 38.3 | 38.3 |
| Mos + Has | 0.0 | 1.7 | 8.3 | 8.3 | 11.7 |  | 0.0 | 1.7 | 8.3 | 8.3 | 11.7 |
| Cor + LI | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Cor + Has | 0.0 | 0.0 | 1.7 | 1.7 | 1.7 |  | 0.0 | 0.0 | 1.7 | 1.7 | 1.7 |
| Kar + LI | 0.0 | 20.0 | 98.3 | 98.3 | 98.3 |  | 0.0 | 20.0 | 98.3 | 98.3 | 98.3 |
| Kar + Has | 0.0 | 23.3 | 90.0 | 91.7 | 91.7 |  | 0.0 | 23.3 | 90.0 | 91.7 | 91.7 |
| Kar + 301 | 8.3 | 11.7 | 95.0 | 100.0 | 100.0 |  | 8.3 | 11.7 | 95.0 | 100.0 | 100.0 |
| Kar + Acx | 6.7 | 10.0 | 88.3 | 100.0 | 100.0 |  | 6.7 | 10.0 | 88.3 | 100.0 | 100.0 |

Ncages/treatment=6; nbees/treatment=60

## **Supplementary Figure S3:** Hazard Ratio Full Trial C

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**Fig S3:** Hazard Ratios (HR) for three insecticide formulations and two spray adjuvants alone or their respective mixtures. All test substances were evaluated against the control within a 72-hour period. Bees from each of the two test colonies were divided into an equal number of cages (i.e. replicate) per treatment. The replicate could thus be evaluated as a covariate as well. (Cox proportional hazard model).

**Supplementary Table S3:** Mortality Data Full Trial C

**Tab S3:** Laboratory test 3 (January 2020): Mortality and corrected mortality M of the control and the 11 treatments up to 72 h after application.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| *Treatment* | *cumulative mortality [%]* | | | | |  | *cumulative corrected mortality M [%]* | | | | |
| *+2* | *+4* | *+24* | *+48* | *+72* |  | *+2* | *+4* | *+24* | *+48* | *+72* |
| C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |  |  |  |  |  |  |
| 133 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Kan | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Mos | 0.0 | 1.7 | 3.3 | 3.3 | 3.3 |  | 0.0 | 1.7 | 3.3 | 3.3 | 3.3 |
| Cor | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Kar | 0.0 | 3.3 | 71.7 | 71.7 | 71.7 |  | 0.0 | 3.3 | 71.7 | 71.7 | 71.7 |
| Mos + 133 | 0.0 | 1.7 | 10.0 | 10.0 | 11.7 |  | 0.0 | 1.7 | 10.0 | 10.0 | 11.7 |
| Mos + Kan | 0.0 | 5.0 | 25.0 | 25.0 | 25.0 |  | 0.0 | 5.0 | 25.0 | 25.0 | 25.0 |
| Cor + 133 | 0.0 | 1.7 | 1.7 | 1.7 | 1.7 |  | 0.0 | 1.7 | 1.7 | 1.7 | 1.7 |
| Cor + Kan | 0.0 | 0.0 | 1.7 | 3.3 | 3.3 |  | 0.0 | 0.0 | 1.7 | 3.3 | 3.3 |
| Kar + 133 | 0.0 | 5.0 | 93.3 | 96.7 | 96.7 |  | 0.0 | 5.0 | 93.3 | 96.7 | 96.7 |
| Kar + Kan | 0.0 | 25.0 | 95.0 | 95.0 | 96.7 |  | 0.0 | 25.0 | 95.0 | 95.0 | 96.7 |

Ncages/treatment=6; nbees/treatment=60

## **Supplementary Figure S4** Hazard Ratio Full Trial D

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**Fig S4:** Hazard Ratios (HR) for two insecticide formulations and two spray adjuvants alone or their respective mixtures. All test substances were evaluated against the control within a 72-hour period. Bees from each of the two test colonies were divided into an equal number of cages (i.e. replicate) per treatment. The replicate could thus be evaluated as a covariate as well. (Cox proportional hazard model).

**Supplementary Table S4:** Mortality Data Full Trial D

**Tab S4:** Laboratory test 4 (August 2020): Mortality and corrected mortality M of the control and the eight treatments up to 72 h after application.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| *Treatment* | *cumulative mortality [%]* | | | | |  | *cumulative corrected mortality M [%]* | | | | |
| *+2* | *+4* | *+24* | *+48* | *+72* |  | *+2* | *+4* | *+24* | *+48* | *+72* |
| C | 0.0 | 0.0 | 0.0 | 0.0 | 1.7 |  |  |  |  |  |  |
| Kan | 0.0 | 0.0 | 0.0 | 0.0 | 1.7 |  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| LI | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |  | 0.0 | 0.0 | 0.0 | 0.0 | -1.7 |
| Siv | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |  | 0.0 | 0.0 | 0.0 | 0.0 | -1.7 |
| Pir | 6.7 | 8.3 | 21.7 | 21.7 | 22.0 |  | 6.7 | 8.3 | 21.7 | 21.7 | 20.7 |
| Siv + Kan | 0.0 | 0.0 | 1.7 | 5.0 | 5.0 |  | 0.0 | 0.0 | 1.7 | 5.0 | 3.4 |
| Siv + LI | 0.0 | 3.3 | 8.3 | 10.0 | 10.0 |  | 0.0 | 3.3 | 8.3 | 10.0 | 8.5 |
| Pir + Kan | 6.7 | 13.3 | 23.3 | 25.0 | 25.0 |  | 6.7 | 13.3 | 23.3 | 25.0 | 23.7 |
| Pir + LI | 10.0 | 18.3 | 31.7 | 33.3 | 33.3 |  | 10.0 | 18.3 | 31.7 | 33.3 | 32.2 |

Ncages/treatment=6; nbees/treatment=60 (Exception: Pir: n=60 until 48 h, from 72 h n=59)

## **Supplementary Figure S5:** Hazard Ratio Full Trial E

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**Fig S5:** Hazard Ratios (HR) for two insecticide formulations and four spray adjuvants alone or their respective mixtures. All test substances were evaluated against the control within a 72-hour period. Bees from each of the two test colonies were divided into an equal number of cages (i.e. replicate) per treatment. The replicate could thus be evaluated as a covariate as well. (Cox proportional hazard model).

**Supplementary Table S5:** Mortality Data Full Trial E

**Tab S5:** Laboratory test 5 (August 2020): Mortality and corrected mortality M of the control and the 14 treatments up to 72 h after application.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| *Treatment* | *cumulative mortality [%]* | | | | |  | *cumulative corrected mortality M [%]* | | | | |
| *+2* | *+4* | *+24* | *+48* | *+72* |  | *+2* | *+4* | *+24* | *+48* | *+72* |
| C | 0.0 | 0.0 | 1.7 | 3.3 | 3.3 |  |  |  |  |  |  |
| 301 | 0.0 | 0.0 | 0.0 | 1.7 | 1.7 |  | 0.0 | 0.0 | -1.7 | -1.7 | -1.7 |
| Acx | 0.0 | 0.0 | 0.0 | 1.7 | 3.3 |  | 0.0 | 0.0 | -1.7 | -1.7 | 0.0 |
| Has | 0.0 | 0.0 | 0.0 | 1.7 | 1.7 |  | 0.0 | 0.0 | -1.7 | -1.7 | -1.7 |
| 133 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |  | 0.0 | 0.0 | -1.7 | -3.4 | -3.4 |
| Siv | 0.0 | 0.0 | 1.7 | 1.7 | 1.7 |  | 0.0 | 0.0 | 0.0 | -1.7 | -1.7 |
| Pir | 5.0 | 6.7 | 13.3 | 13.3 | 13.3 |  | 5.0 | 6.7 | 11.9 | 10.3 | 10.3 |
| Siv + 301 | 6.7 | 8.3 | 15.0 | 16.7 | 16.7 |  | 6.7 | 8.3 | 13.6 | 13.8 | 13.8 |
| Siv + Acx | 0.0 | 1.7 | 1.7 | 1.7 | 1.7 |  | 0.0 | 1.7 | 0.0 | -1.7 | -1.7 |
| Siv + Has | 0.0 | 0.0 | 5.0 | 5.0 | 6.7 |  | 0.0 | 0.0 | 3.4 | 1.7 | 3.4 |
| Siv + 133 | 0.0 | 11.7 | 16.7 | 18.3 | 18.3 |  | 0.0 | 11.7 | 15.3 | 15.5 | 15.5 |
| Pir + 301 | 10.0 | 35.0 | 58.3 | 60.0 | 65.0 |  | 10.0 | 35.0 | 57.6 | 58.6 | 63.8 |
| Pir + Acx | 15.0 | 18.3 | 40.0 | 41.7 | 41.7 |  | 15.0 | 18.3 | 39.0 | 39.7 | 39.7 |
| Pir + Has | 8.3 | 20.0 | 48.3 | 50.0 | 50.0 |  | 8.3 | 20.0 | 47.5 | 48.3 | 48.3 |
| Pir + 133 | 6.7 | 15.0 | 38.3 | 40.0 | 40.0 |  | 6.7 | 15.0 | 37.3 | 37.9 | 37.9 |

Ncages/treatment=6; nbees/treatment=60