

1.17 Comparison of Control and Toxic Reference Data between Honey Bee Laboratory Studies Conducted in Germany and in Spain over the Last Decade

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Abstract

The Draft EFSA Bee Guidance Document (2013) describes various types of bee studies to be part of the risk assessment. Eurofins Agrosience Services (EAS) has been conducting acute toxicity and chronic feeding studies with adult bees over the last decade and larval acute and chronic studies over the last 5 years in Germany and in Spain. The studies are conducted with different subspecies and in different times of the year in the two countries.

The aim of the comparison is to find out if season and geographical origin of the bees have any influence on the test outcome, i.e. control/solvent control mortality and reference item 24 h LD₅₀ range with controls and toxic reference data collected over multiple years. The results give an indication how relevant the testing of different subspecies is for the registration of plant protection products in Europe.

Keywords: Honey bee, oral and contact acute toxicity, chronic feeding test, larval test, control, toxic reference, Germany, Spain

Introduction

Acute oral and contact toxicity tests with the honey bee, *Apis mellifera* (OECD TG 213 and 214, 1998) have been part of the risk assessment of plant protection products (PPP) for decades. Since the release of the Draft EFSA Bee Guidance Document (2013), adult chronic feeding studies and larval acute and chronic studies are also part of the risk assessment. Eurofins Agrosience Services (EAS) has been conducting acute toxicity and chronic feeding studies with adult bees over the last decade and larval acute and chronic studies over the last 5 years in Germany, region Baden Württemberg, and in Spain, region Valencia. The studies in Germany are conducted with Central European Bees *Apis mellifera carnica* Pollmann from April until September, whereas in Spain they are conducted with *Apis mellifera* L. from September until June.

Experimental Methods

Test Organisms and Test Period:

In Germany honey bee tests are conducted with *Apis mellifera carnica* Pollmann from mid-April until mid-September, when foraging and egg laying activities are at their peak. In Spain tests are conducted with *Apis mellifera* L. all year round, except during extreme heat periods (July/August).

Acute Toxicity Test:

In Germany the test organisms are collected from the honey chamber and introduced into the test units without anaesthetization. In Spain the test organisms are collected from the outer combs of the bee hive and briefly anaesthetized with CO₂ before introduction in the test units.

After a 24h acclimatization period the oral or contact exposures are conducted in the same manner in both countries, under identical climatic conditions following the OECD TG 213 and 214, 1998.

Chronic Feeding Test:

In Germany and Spain brood combs are collected from the bee hives and transported to a climatic chamber. Freshly hatched bees are introduced in the test units without anaesthetization. After a 24 h acclimatization period freshly prepared (treated) feeding solutions are offered to the 1 to 2 days old bees for 10 days, under identical climatic conditions following the OECD TG Proposal, 2016. Several solvents can be used (acetone up to 5 %, acetone + 0.1 % xanthan, acetone + 0.1 % xanthan + 1 % Tween80).

Larval Chronic Test:

In Germany and Spain, synchronised honey bee larvae are transferred into well-plates and reared under identical climatic conditions. The larvae are fed with standardized amounts of an artificial (treated) diet. The chronic test is conducted following the OECD Guidance Document 239 (2016) without the use of emergence boxes between day 15 and day 22. Acetone is used as solvent at the maximum concentration of 0.5 %.

Results

Acute Toxicity Test

For acute toxicity testing no statistically significant differences were found between the control mortality in Germany and in Spain (Mann-Whitney U test, $p \geq 0.05$). However, the 24 h oral and contact LD₅₀ values are statistically significantly different. The German subspecies seems to be slightly more sensitive in oral toxicity testing than the Spanish subspecies. However, in contact toxicity testing the LD₅₀ in Spain was lower. One reason for the result could be the additional anaesthetization conducted in Spain before introduction of the test organisms into the test units. Nevertheless, the mean LD₅₀ values were very similar (mean oral LD₅₀ 0.13 and 0.12 µg dimethoate/bee and mean contact LD₅₀ 0.15 µg and 0.17 µg dimethoate/bee for Spain and Germany, respectively). See Figure 1.

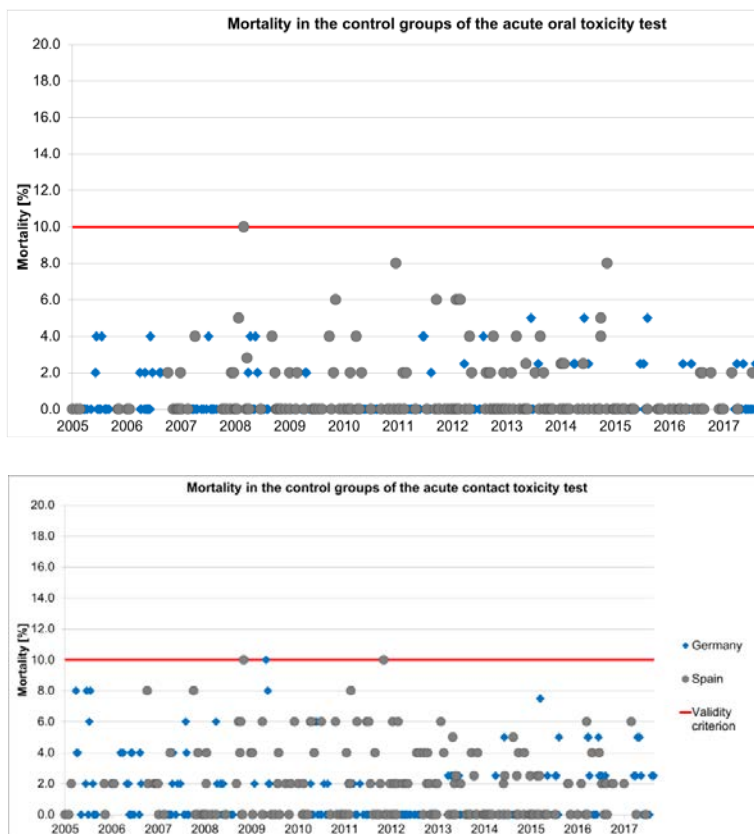


Figure 1 Control mortality in the acute toxicity tests

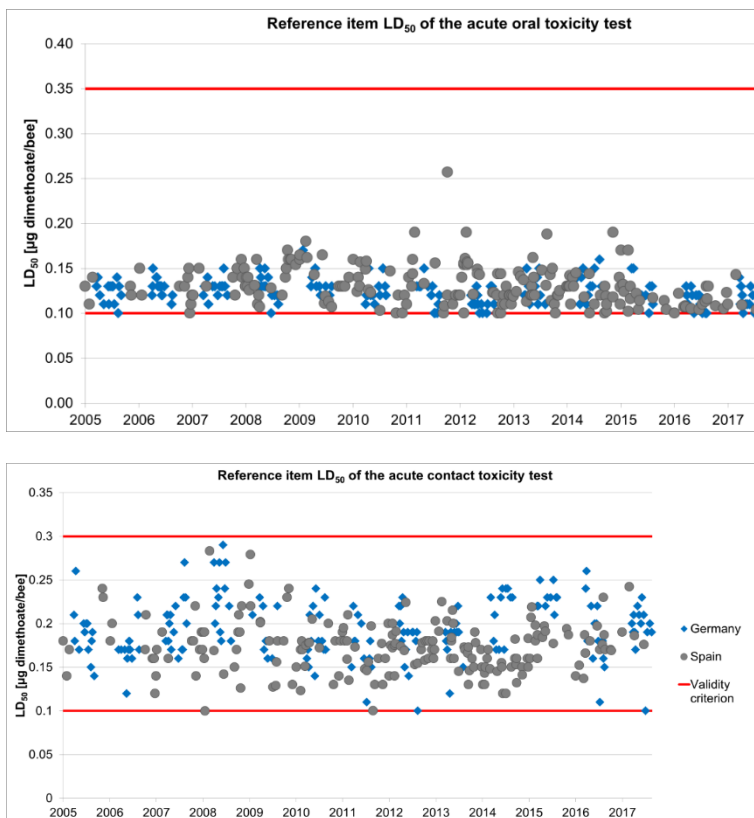
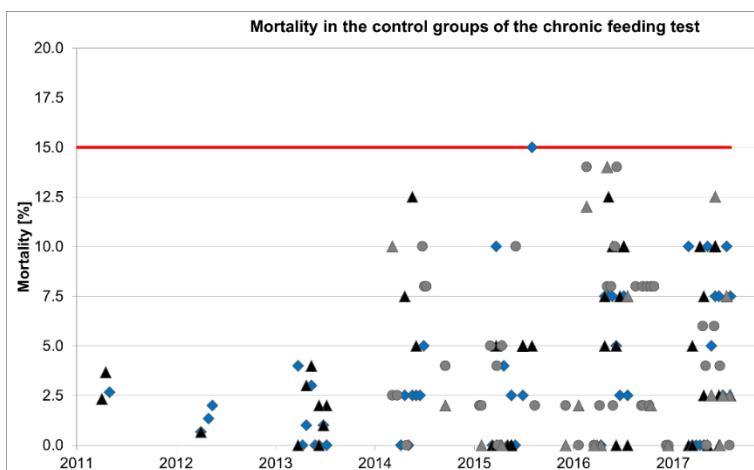


Figure 2 Reference item mortality in the acute toxicity test

Chronic Feeding Test

For chronic feeding testing no statistically significant differences (Mann-Whitney U test, $p \geq 0.05$) were found between Germany and Spain when comparing control, solvent controls or reference item mortality.



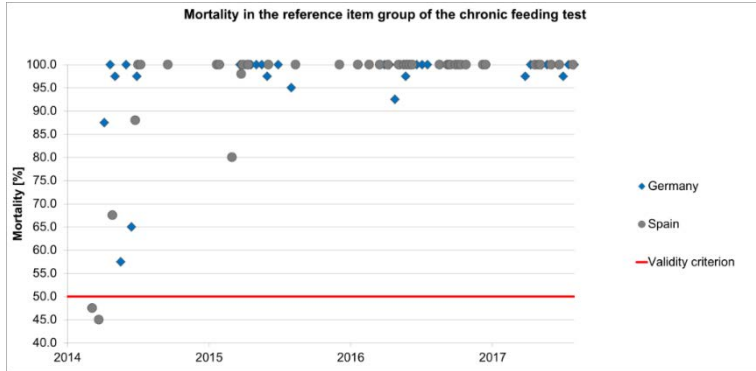
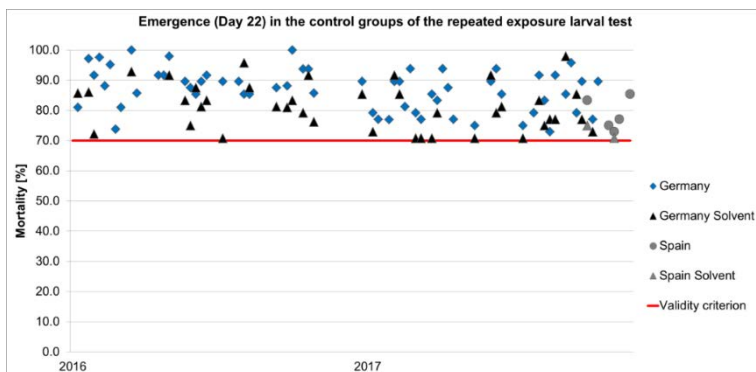
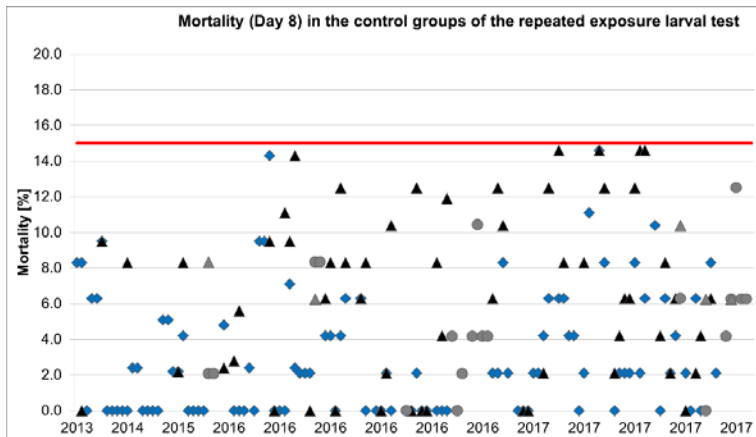


Figure 3 Control and reference item mortality in the chronic feeding test

Larval Chronic Test

For larval chronic testing the toxic reference mortality between Germany and Spain is statistically significantly different (Mann-Whitney U test, $p \geq 0.05$), showing a higher sensitivity in Germany.



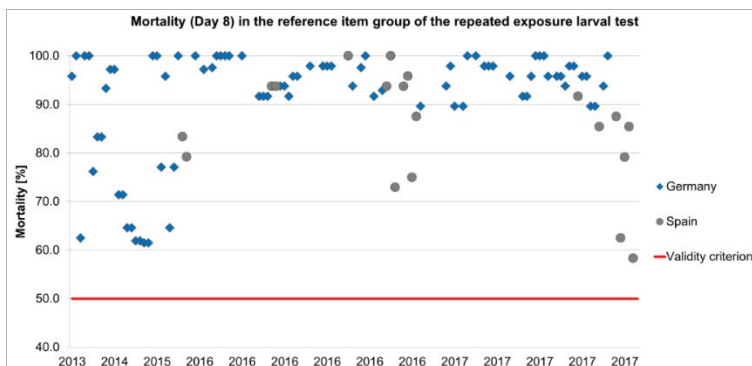


Figure 4 Control and reference item mortality and control emergence in the larval chronic test

Conclusions

The control/solvent control data prove that control/solvent control mortality is in the same range for Germany and Spain for all test systems. The differences of reference item data in acute oral toxicity and larval chronic testing indicate a slightly higher sensitivity under German conditions (subspecies, season and geographic origin) compared to Spanish conditions. However, the differences have no influence on the validity of the test. Differences found here are very small and provide evidence that the test systems are robust. It can be concluded that the season, the geographical origin and the different subspecies have little relevance for the registration of plant protection products in Europe.

References

- OECD TG 213: Honeybees; acute oral toxicity test (OECD 1998)
- OECD TG 214: Honeybees; acute contact toxicity test (OECD 1998)
- OECD TG Proposal: Honey bee (*Apis mellifera*, L.), chronic oral toxicity test (10-day feeding) (OECD 2016)
- OECD 239: Honey Bee (*Apis mellifera*) Larval Toxicity Test, Repeated Exposure (OECD 2016)