Statement on the findings of the study:

Lu et al. (2012): In situ replication of honey bee colony collapse disorder

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Contents of the publication

In their publication "In situ replication of honey bee colony collapse disorder", LU et al. (2012) describe a trial in which they claim they have replicated inducing symptoms of Colony Collapse Disorder (CCD) by chronically exposing bee colonies to high-fructose corn syrup (HFCS) contaminated with Imidacloprid, and bring forward a new hypothesis about the origin of CCD. The authors hypothesize that CCD is caused by Imidacloprid residues originating from seed treatment in corn in HFCS which is used to feed honeybee colonies. In order to verify this hypothesis, they set up the following trial design: In each of four different apiaries, five commercial colonies were set up, four were fed with HFCS containing Imidacloprid at different concentrations, the fifth received uncontaminated HFCS and served as control colony. The colonies of the treatment groups were first exposed to a lower Imidacloprid concentration for 4 weeks, followed by a higher exposure concentration for subsequent 9 weeks. The exposure concentrations of the different treatment groups were 0.1 + 20 µg/kg, 1.1 + 40 µg/kg, 5.3 + 200 µg/kg, and 10.5 + 400 µg/kg. Treatments were repeated weekly between July and September 2010. In this period, hives were assessed weekly, with bi-weekly brood assessments. Further weekly hive assessments followed in the time period between December 2010 and March 2011. At the end of the assessment period all colonies of the two higher treatment group and the 20 µg/kg treatment group, three out of four colonies of the 40 µg/kg group, and two out of four colonies of the control group were found dead. From this observation, the authors conclude that the observed mortality, which they attribute to exposure to Imidacloprid, demonstrate that Imidacloprid residues in HFCS are a plausible mechanism to explain CCD.

Comments from the BCS perspective

It is obvious that the study design applied by LU et al. (2012) suffers from many flaws, with regard to the set-up of the trial as well as regarding the context in which it was conducted. Moreover, many of the conclusions drawn from the findings are highly questionable. In the following, the most critical points are listed and discussed.

Trial Design – Replication. In ecotoxicological bee testing, especially when entire honeybee colonies are subject of the test, the choice of a suitable design is of key importance for obtaining scientifically solid results. An important aspect of this is an appropriate replication, especially since a bee colony is a highly complex meta-organism, a fact which entails a significant natural variability in almost all testing endpoints that may be measured in such a study. The numbers of replicates that are needed to appropriately address a certain endpoint depend largely on the complexity and intrinsic variability of the endpoint to be measured. For a relatively simple endpoint like mortality or foraging activity, relatively low replicate numbers like three or four, as normally used in semi-field tests, are commonly considered appropriate. However, this cannot be applied to the study under discussion here. One of the most complex endpoints one could think of is overwintering success and overwintering mortality of bee colonies – an endpoint that depicts the upshot of a long, complex process that is influenced by numerous, partly interdependent variables and factors, many of which are not even known to us. To address this kind of endpoint with a relatively low number of replicates, as Lu et al. (2012) did, (only four hives per treatment group!) seems hardly appropriate. Therefore it is questionable in how far their design is at all capable of detecting significant differences between different experimental groups in the endpoint overwintering mortality. The authors seem to be aware of this as they call this weakness of their study design an "apparent deficiency". It remains the question then: why do they nevertheless draw conclusions from a test conducted under an obviously flawed design? In this context, it is likewise notable that the authors apparently did not conduct a statistical analysis of their colony mortality data, as would be expected in a solid scientific study.

In addition to the low numbers of colonies of the individual treatment groups that actually do not allow drawing conclusions from the outcome of the study, similarly the dose-response relationship in colony mortality that the authors claim to see, is rather doubtful: in the 20 µg/kg exposure group, mortality set in earlier than in the 40 µg/kg as well as in the 200 µg/kg treatment group, and reached higher levels than in the 40 µg/kg group. This is not consistent with the assumption of a dose-response relationship. Likewise, the authors state that they have "found that the initial brood rearing corresponded to imidacloprid doses two weeks after the initial imidacloprid dosing, however, it is inversely related to imidacloprid dosages at the end of dosing regime", however, they then admit that the "decrease [of brood cells during the observation period] is independent of different Imidacloprid doses applied to the hives". According to the graph sown in Figure 1, there is no obvious or significant dose-response relationship in the abundance of brood cells at all – values fluctuate, as typical for this endpoint, over time, with sometimes the control group, sometimes a treatment group (in particular the 20 µg/kg and the 40 µg/kg groups) having highest abundances of brood cells. Towards the end of the season, brood abundance decreases in all treatment groups, which is a natural phenomenon related to the generation cycle of a bee colony. During this period, measurements of brood abundance will anyway lead to erratic figures, so there is no sense in making comparisons between observations on the development of this endpoint in different treatment groups during this time of the year.

Another point that raises some doubt that the test design as used by Lu et al. (2012) would be capable of reliably detecting treatment effects and yielding anything else but erratic results, is the following: there is no doubt that dietary Imidacloprid concentrations of 400 or even of 200 µg/kg are hazardous to bees, especially when chronically administered to a colony. When a colony is exposed to such concentrations over many weeks, one should expect that there would be effects of this visible. In so far it is surprising that apparently no such effects were seen at all during all the exposure period, and that first signs of "treatment-related" colony mortality only set in 13 weeks after exposure. This suggests that the test design was not sufficiently sensitive to detect any treatment-related effects, and the distribution of the observed mortality over the different treatment groups was just erratic.

Exposure of Bee Colonies to Neonicotinoids. In a sound scientific approach, it would have been the logical procedure to first gather information about the nature of a realistic exposure scenario, before simulating this scenario in a trial. In the given case, this would have meant evaluating what residue levels of Imidacloprid would normally be present in HFCS, and then defining the exposure levels to be tested in a study on the basis of these realistic exposure levels. However, apparently the authors had no information available about potential residue levels in HFCS, and instead of determining a realistic exposure scenario by conducting residue analyses in commercially available HFCS samples, they based the exposure levels tested in their study on pure guesses which are completely unsubstantiated. They argue that the maximum residue level of Imidacloprid in corn as set by US EPA is 50 µg/kg. As there is no maximum residue level set for HFCS, they arbitrarily assume that residue levels in HFCS are the ten-fold maximum tolerated level for corn grain, from which HFCS is processed. First, deducing an anticipated, common environmental exposure concentration from a maximum tolerated residue level is absurd and not in compliance with any scientifically sound principle. Second, a ten-fold extrapolation factor for a product which is even not processed in a concentration process (see below) is by no means justified (analogy: this would be like assuming that each pedestrian walking on the sidewalk along a street with a 100 km/h speed limit would move there with a speed of 1000 km/h – the speed limit is basically valid for cars, so other entities may move at much higher speeds, even though common sense suggests that they would not do so).

Another point which is very questionable related to the author's assumption that there were significant Imidacloprid residues in HFCS refers to the following consideration: if their assumption was true that there is widespread occurrence of residue levels of Neonicotinoids in HFCS, why did they then not analyze the HFCS they used for Neonicotinoid residue levels just to determine these levels, and why did they then have to spike it with Imidacloprid at all – if their hypothesis was correct, then residues of the compound would already have been in there, and spiking it again would have been double dosing. Then, if there would be significant residue levels of Imidacloprid in commercially available HFCS, why did the authors then find no residues in their analysis of the blank sample fed to their control hives (Table 2)? Implicitly, the authors seem to suggest that a problem with Imidacloprid residues in HFCS must have been in

particular in older batches produced in 2005/06, as they point out that they were not able to obtain HFCS manufactured during this years, so they "used food-grade HFCS fortified with different levels of Imidacloprid, mimicking the levels that are assumed to have been present in the older HFCS." This approach is lacking any logical coherence – if a significant residue level of Imidacloprid was present in HFCS in 2005/06, but no more nowadays, how can the incidences of CCD symptoms that are still reported in more recent years, be attributed to Imidacloprid residues in HFCS? Moreover, the authors give no explanation why in the framework of their hypothesis Imidacloprid residues in HFCS should have been different between 2005/06 and nowadays.

Then, even if there were Imidacloprid residues in HFCS as the authors hypothesize, the exposure scenario as tested would not be realistic: in apicultural practice, no bee colony would be fed with HFCS all season long. In so far, exposure conditions as applied here would be strongly exaggerated in any case.

Whatever assumptions about residues of Neonicotinoids may be present in HFCS might have been made by the authors, it seems, in contrast to their hypotheses, to be a matter of fact that HFCS contains no Neonicotinoid residues at all. In 2009, USDA analyzed 12 HFCS samples of different origins for pesticide residues; these residue analyses screened for ca. 200 active ingredients; the detection limit for Imidacloprid was 1 µg/kg. No residues of Imidacloprid or other Neonicotinoids were found (Roger Simonds, USDA Agrimarketing Service, Gastonia, North Carolina, personal communication). Therefore, it seems very unlikely that residues of Imidacloprid or other Neonicotinoids are prevalent in HFCS.

That no Imidacloprid residues are found in HFCS that is produced from seed-treated corn is not surprising when considering the process in which this commodity is usually produced: Corn grain is converted to corn syrup with several production steps. First, the grain is wet milled to produce corn starch. The starch is taken through three separate enzymatic conversions to break the starch into oligosaccharides, then to hydrolyze the oligosaccharides to glucose, and finally to isomerize the glucose to a glucose/fructose mixture. The raw corn syrup is then purified with activated carbon. Even if Imidacloprid residues that might potentially be present in the grains would not be degraded in the maceration process, they would ultimately be removed from the syrup by final activated carbon purification. Moreover, there is no step in this production process in which Imidacloprid could be concentrated. Therefore, even if there would be residues in HFCS, they would be lower rather than higher than those that might be found in corn grain.

Another point to justify the tested concentrations that the authors are bringing forward is a reference to a paper of GIROLAMI et al. (2009) who found Imidacloprid residue levels of 47 mg/kg in guttation liquid from seedlings of Imidacloprid seed-treated maize plants. It is not in any way reasonable to compare residue levels in xylem liquid of young seedlings (this is where guttation fluid originates from) with residue levels in processed products originating from fruits from mature plants in which the substance used for seed treatment has been massively diluted and degraded. Moreover, it has been convincingly demonstrated that guttation liquid is not normally used by bee colonies as relevant water source (KEPPLER et al. 2010, PISTORIUS et al. in press), and even in cases where individual bees might be picking up guttation fluid, this would not lead to a long-term exposure of the colonies as it was in the design of the study of LU et al.

(2012). The authors state then that "the finding of the loss of honey bee hives at the levels as low as 20 µg/kg of imidacloprid in HFCS raises the question of whether there is a no observed- adverse-effect-level of imidacloprid (and most likely of other neonicotinoids as well) for honey bees." This statement is erroneous: A NOAEC for Imidacloprid has been established in numerous honeybee studies under field-relevant conditions (see for instance MAUS et al. 2003, SCHMUCK et al. 2005). That the authors were not able to determine a NOAEC in their study may on one hand be related to the fact that the described experiment was probably not capable of establishing a robust NOEC for the endpoint overwintering mortality due to flaws of the design (see above). On the other hand, the authors tested, compared to environmental exposure levels, excessively high dietary concentrations: even the lowest exposure level tested (20 µg/kg) is by far higher than the residue levels that are normally found in nectar and pollen of seed-treated crops (see for instance MAUS et al. 2003, SCHMUCK et al. 2005), and the highest concentrations tested by LU et al. (2012) were hundred-fold and more overdosed compared to typical environmental concentrations.. The authors argue that the bees of the test colonies would have reduced the concentration of Imidacloprid in their food supply by diluting the offered HFCS with nectar collected from nearby floral resources, but to reach realistic exposure levels, even the lowest test concentration would have to be diluted by a factor of four to ten, which is, under consideration of the season when exposure took place (end of July to end of September when natural food sources for bees tend to be scarce), highly unlikely. A chronic dietary exposure to residue levels as high as 20 µg/kg, as tested in the study under discussion here is therefore a scenario that would not occur under realistic agricultural conditions. Interestingly, in an earlier study on overwintering success of bee colonies exposed to Imidacloprid (FAUCON et al. 2005), where environmentally more relevant residue levels (0.5 and 5 µg/kg) were tested with more appropriate replicate numbers (8 to 9 per treatment group), no adverse effects to the exposed hives were found.

Another debatable point regarding the dosing regimes applied in the study under discussion here is why the authors first applied low exposure concentrations between 0.1 and 10.5 µg/kg which, at least the lower concentrations, may come close to what a bee colony may be exposed to in the field, but then after a few weeks switched to at least partly dramatically exaggerated rates. There seems no be no reasonable rationale behind this, and the authors likewise do not justify this unusual dosing regime.

Correlations between colony mortality and the use of Neonicotinoids. The authors claim that the first significant occurrence of CCD in US in 2006/07 was correlated with the introduction of neonicotinoid insecticides as seed treatment in corn in 2004/05. This, again, is a statement which is based on several misconceptions and errors: 1.) Imidacloprid as a seed treatment in corn was brought on the market in US in 2000, and was first used there during the planting season 2001. 2.) Imidacloprid seed treatment in corn was initially only applied on a very limited acreage in US, and after two years on the market, it was hardly used any more, being replaced by other products. During the years 2004 to 2011, the percentage of US corn acres treated with Imidacloprid has been less than one-half of one percent (BCS, unpublished data).

Therefore it is ridiculous to assume that after market introduction of Imidacloprid in corn, Imidacloprid residues might have been prevalent in HFCS produced in US, and the claimed correlation is completely unsubstantiated.

If there would exist a causative link between the use of Neonicotinoids and honeybee colony mortality, one should see a correlation between use of Neonicotinoids and exposure of bees to Neonicotinoids on one hand, and colony losses on the other hand. However, this is not the case at all. On the contrary, CCD occurrence and other colony losses that have been observed at large scales are not correlated with exposure of honey bee colonies to Neonicotinoids (VAN ENGELSDORP et al. 2009, DELAPLANE 2012) or to exposure of colonies to Neonicotinoid-treated crops (e.g. OTTEN 2003a, b, CHARRIÈRE & NEUMANN 2010). Linkage of Neonicotinoid exposure to declining bee colony health and elevated colony losses has not been found in any of the recent regional multifactorial studies of declining bee health (VAN ENGELSDORP et al. 2009, 2010a, ROGERS & KEMP 2004, NGUYEN et al. 2009, CHAUZAT et al. 2009, GENERSCH et al. 2010). As another example, no unusual colony losses are reported from Australia, where a lot of Neonicotinoid seed treatment is applied (but no *Varroa* mites are present) (NEUMANN & CARRECK 2010). Moreover, in recent scientific reviews of the evidence for whether Neonicotinoid pesticides play a causal role in bee declines (BLACQUIERE et al. 2012, CRESSWELL et al. 2012), the conclusion reached is there is no evidence that they do. Finally, if increased colony mortality would in fact be caused by Neonicotinoid-contaminated HFCS, how could then the observed colony losses in regions where HFCS is or has not been not commonly used to feed bee colonies (like Europe, Asia) be explained?

Symptoms of mortality. The authors claim to have induced CCD in their study. However, the symptoms described do not seem to support this claim. In late December 2010, all colonies were found alive, but the ones exposed to higher levels of Imidacloprid "appeared" weaker with smaller bee clusters inside the hives (apparently just an estimate), and dead bees were found in front of the hives. Then subsequently, more and more colonies died after these signs of weakening. The dead hives were "remarkably empty", just food stores with honey and pollen were left in the combs. VAN ENGELSDORP et al. (2009) describe in detail the symptoms used to define colonies as suffering from CCD. Symptoms include (1) "the apparent rapid loss of adult worker bees from affected colonies as evidenced by weak or dead colonies with excess brood populations relative to adult bee populations, (2) a noticeable lack of dead worker bees both within and surrounding the affected hives, (3) the delayed invasion of hives pests (e.g. small hive beetles and wax moths), and kleptoparasitism (stealing food) from neighboring honey bee colonies". Other authors (summarized by HENDRIKX et al. 2009) add that CCD is furthermore characterized by a sudden disappearance of worker bees during the beekeeping season, and by evidence of recent brood (young larvae, seemingly healthy queen). Almost none of these symptoms was observed in connection with the colony mortality described by LU et al. (2012): the disappearance and the collapse of the affected colonies did apparently not set in spontaneously, but was preceded by a weakening of the colonies; moreover, dead bees were found in front of the hive, which would by definition not be the case in incidences of CCD,

but which is normal for overwintering colonies. There is no mention of the presence of a surviving queen in the affected hives, but it can be implied that the authors would have recorded the presence of a lonesome queen in the "remarkably empty" hives. Moreover, there is no information provided about the presence of bee brood, but since the observation were made during winter time, it can be assumed that there was no brood. Thus, what the authors have seen was colony mortality, but certainly not CCD.

Potential causes of mortality. The authors claim that "The loss of imidacloprid-treated hives in this study is also highly unlikely due to pathogen infection since the presence of neither *Nosema* nor a large number of *Varroa* mites was observed in hives during the summer and fall seasons.", and they conclude that "Since all hives were considered healthy as they went into fall season, those pathogens posed very little threat to the health of honey bee hives." They substantiate this by an Apistan® (active ingredient: Tau-Fluvalinate) and Fumagillin B treatment against *Varroa* and *Nosema* that had been conducted in October. Apparently, however, the health status of the colonies and the presence or absence of parasites was not accurately checked with appropriate methods (or, if so, the results of this health analysis, like *Varroa* counts or *Nosema* spore counts, are not documented), and it appears that hives were simply declared healthy by the authors based on the lack of obvious signs of disease or parasite infestation seen by visual inspection. However, the absence of conspicuous signs of diseases and parasites in late summer is by no means conclusive evidence that the hives were not infested at all. Many beekeepers who have lost their hives over winter due to Varroosis or other diseases would have considered them healthy when visually inspecting them in late summer or fall. Likewise, just the fact that a treatment against a certain parasite has been conducted does unfortunately by no means guarantee that the treatment was successful and that the treated hive was and has remained parasite-free later on, especially when the treatment was conducted with an inappropriate timing (see below), and with an active ingredient that might be not efficient, if possible resistance has not been checked. For instance, according to OTTEN (2005), German apiaries which had been treated with Apistan® had losses as high as 43% over the winter of 2002/03. As the authors likewise do not provide any data about diagnosis of diseases or parasites in the colonies that died over the winter, it is very possible that the observed mortality was caused by *Varroa* or other parasites or diseases which are commonly recognized to be the main factor behind colony overwintering mortality.

Hive management. In the publication of LU et al. (2012) there are some details described about the handling of the study colonies that give rise to the assumption that they have been improperly managed. For instance, *Varroa* treatment with Apistan® was conducted in October, which is much too late in the season to be fully effective. Moreover, the applied product is not effective against mites in many areas of the US. It is very possible that these management practices have contributed to the overall high colony mortality observed in this study. Another point to mention is that the hives were apparently opened once a week for assessments, even during the winter months at low temperatures. This is certainly not beneficial

for colony health and may likewise have weakened them. In this context it is notable that hive management is in fact an important factor causing colony losses – VAN ENGELSDORP et al. (2010b) even rank it higher than CCD as a factor causing colony mortality.

Errors. Finally, there are several statements in the paper that are simply untrue or incorrect in the given context. These include:

"The abrupt emergence of colony collapse disorder (CCD) in the United States during 2006-2007, and other countries later has raised the concern of losing this important perennial pollinator globally." - Large-scale losses are not new to the beekeeping industry. Many of the symptoms similar to those related to CCD have been described before. The first published record of this disorder appeared already in 1869. Subsequently losses were described in Colorado in 1891 and 1896 where large clusters disappeared or dwindled to tiny clusters with queens in May, hence the name "May disease" (UNDERWOOD & VAN ENGELSDORP 2007). Then, CCD is not a common, global phenomenon, but specific to USA; in Europe, for instance, colony losses with CCD symptoms are exceptional (see for instance HENDRIKX et al. 2009, VAN ENGELSDORP et al. 2009, VAN ENGELSDORP & MEIXNER 2010). Even in the USA, many professional apiarists have never seen a single case of CCD. The vast majority of colony losses in the US are not from CCD (see e.g. VAN ENGELSDORP et al. 2010b).

"Although some losses of honey bees from healthy and well managed hives during the winter months have always been part of apiculture (for instance, in the New England area, winter losses of honey bee hives are typically 15-30%), never in the history of the beekeeping industry has the loss of honey bee hives occurred in such magnitude and over such a widely distributed geographic area." – Sadly enough, beekeepers are not facing only "some losses" and certainly not limited to the New England area. According to NEUMANN & CARRECK (2010) elevated colony losses have recently been reported from e.g. Europe (CRAILSHEIM et al., 2009), the USA (VAN ENGELSDORP et al., 2009; 2010) and the Middle East (HADDAD et al., 2009, SOROKER et al., 2009) but only rarely from South America and virtually not at all from Africa and Australia. The scientists cited above linked this to the fact that colonies of African honey bees and Africanized honey bees in South America survive without *Varroa* treatment, whilst the mite has not yet introduced into Australia. Furthermore, bee colony losses are clearly not a new phenomenon, they just gained more attention: VAN ENGELSDORP & MEIXNER (2010) examined the historical records and showed that extensive losses are not unusual. Already almost a century ago, in 1906, beekeepers on the Isle of Wight (England), noticed that many of their honey bee colonies were dying, with numerous bees crawling from the hive unable to fly. Older records report massive bee colony losses without obvious disease symptoms from Australia in 1872 (BEUHNE 1910).

"Winter losses of honey bee hives usually occur because honey bees run out of or cannot access food, or the cluster becomes too small to generate sufficient heat." – Starvation is one of the potential causes of overwintering mortality, however, clearly not the only or even not the most predominant one. Factors involved in overwintering mortality are for instance listed by VAN ENGELSDORP et al. (2010b) and by BRODSCHNEIDER et al. (2010).

"Commercial beekeepers appear to be affected by CCD at a disproportional rate" – According to van ENGELSDORP et al. (2010b), professional beekeepers record on average less pronounced losses compared to small apiaries.

To explain that mortality only set in several months after exposure to the treatment, the authors hypothesize that winter bees were in their larval stage exposed to Imidacloprid, and therefore may have been sublethally affected by this as adults. As an evidence for this kind of phenomenon, they cite the publication of MEDRZYCKI et al. (2010). However, this paper describes different sensitivities of bees that were reared at different temperatures to an insecticide, and not a reduced vitality of bees that were reared exposed to a pesticide. In so far, this study is not of relevance in the given context.

Conclusions

The study presented by Lu et al. (2012) has been conducted according to a faulty design that was based on numerous incorrect to unsupportable assumptions which are totally inconsistent with a sound scientific approach. The results are accordingly insignificant for any risk assessment, moreover the authors interpret them in a very questionable way. The study provides no evidence at all that the exposure of honeybee colonies to Neonicotinoids under realistic conditions might have any adverse effect.

References

BEUHNE, R. (1910): Bee mortality. - Journal of the Department of Agriculture of Victoria, Australia **8**: 149-151

BLACQUIÈRE, T., G. SMAGGHE, C.A.M. VAN GESTEL & V. MOMMAERTS (2012): Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment. – Ecotoxicology: DOI 10.1007/s10646-012-0863-x

BRODSCHNEIDER, R., R. MOOSBECKHOFER & K. CRAILSHEIM (2010). Surveys as a tool to record winter losses of honey bee colonies: a two year case study in Austria and South Tyrol. - Journal of Apicultural Research **49**: 23-30

CHARRIÈRE, J.D. & P. NEUMANN (2010): Survey to estimate winter losses in Switzerland. – Journal of Apicultural Research **49**: 132-133

CRAILSHEIM, K., R. BRODSCHNEIDER& P. NEUMANN (2009): The COLOSS puzzle: filling in the gaps. - In: Proceedings of the 4th COLOSS Conference, 3-4 March 2009. Zagreb, Croatia: 46-47.

CHAUZAT, M.P., P. CARPENTIER, A.C. MARTEL, S. BOUGEARD, N. COUGOULE, Ph. PORTA, J. LACHAIZE, F. MADEC, M. AUBERT & J.P. FAUCON (2009) : Influence of Pesticide Residues on Honey Bee (Hymenoptera: Apidae) Colony Health in France. - Environmental Entomology **38**: 514-523

CRESSWELL, J.E., N. DESNEUX & D. van ENGELSDORP (2012): Dietary traces of Neonicotinoid pesticides as a cause of population declines in honey bees: an evaluation by Hill's epidemiological criteria. - Pest Management Science: doi: 10.1002/ps.3290.

DELAPLANE, K. (2012): Managed Pollinator Coordiated Agricultural Project Highlights and Emerging Trends. - Bee Culture **12**: 24-25.

GENERSCH, E., W. von DER OHE, H. KAATZ, A. SCHROEDER, Ch. OTTEN, R. Büchler, S. BERG, W. RITTER, W. MÜHLEN, S. GISDER, M. MEIXNER, G. LIEBIG & P. ROSENKRANZ (2010): The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies. - Apidologie **41**: 332-352

FAUCON, J.P., C. AURIERES, P. DRAJNUDEL, L. MATHIEU, M. RIBIÈRE, A.C. MARTEL, S. ZEGGANE, M.P. CHAUZAT & M.F.A. AUBERT (2005): Experimental study on the toxicity of imidacloprid given in syrup to honey bee (*Apis mellifera*) colonies. - Pest Management Science **61**:111–125

GIROLAMI, V., M. MAZZON, A. SQUARTINI, N. MORI, M. MAZARO, A. DI BERNARDO, M. GREATTI, G. GIORIO & A. TAPPARO (2009): Translocation of neonicotinoid insecticides from coated seeds to seedling guttation drops: a novel way of intoxication for bees - Journal of Economic Entomology **102**: 1808-1815.

HADDAD, N., A. BATAENEH, I. ALBABA, D. OBEID & S. ABDULRAHMAN (2009): Status of colony losses in the Middle East. - In: Proceedings of the 41st Apimondia Congress, Mointpellier, France:.36

HENDRIKX, P., M.-P. CHAUZAT, M. DEBIN, P. NEUMANN, I. FRIES, W. RITTER, M. BROWN, F. MUTINELLI, Y. LE CONTE & A. GREGORC (2009): Scientific Report Submitted to EFSA : Bee Mortality and Bee Surveillance in Europe.: 217 pp.

KEPPLER, J., R. BECKER, R. SPATZ & F. DEchet (2010): Systemische insektizide Beizmittel – Auftreten und Relevanz von Guttation für die Entwicklung von Honigbienenkolonien - Julius-Kühn-Archiv 428: 133

MAUS, Ch., G. CURÉ & R. SCHMUCK (2003): Safety of imidacloprid seed dressings to honey bees: a comprehensive overview of compilation of the current state of knowledge. - Bulletin of Insectology **56**: 51-57.

NEUMANN, P. & N.L. CARRECK (2010): Honey bee colony losses. - Journal of Apicultural Research **49**: 1-6

MEDRZYCKI, P., F. SGOLASTRA, L. BORTOLOTTI, G. BOGO, S. TOSI, E. PADOVANI, C. PORRINI & A.G. SABATINI (2010): Influence of brood rearing temperature on honey bee development and susceptibility to poisoning by pesticides - Journal of Apicultural Research **49**: 52-59.

NGUYEN, B.K., C. SAEGERMAN, G. PIRARD, J. MIGNON, J. WIDART, B. THIRONET, F.J. VERHEGGEN, D. BREKVENS, E. DE PAUW & E. HAUBRUGE (2009): Does Imidacloprid Seed Treated Maize Have an Impact on Honey Bee Mortality? – Journal of Economic Entomology. **102**: 616-623

OTTEN, Ch. (2003a): Das Völkersterben: Daten und Fakten. – Deutsches Bienen Journal 8/2003: 312-314

OTTEN, Ch. (2003b): Daten und Fakten zu den Völkerverlusten. – ADIZ 8/2003: 6-8

OTTEN, Ch. (2005): Bee Colony Mortality in Germany: Data and Facts. - – In: FORSTER, R., E. BODE & D. BRASSE (eds.): Das "Bienensterben" im Winter 2002/2003 in Deutschland – Zum Stand der wissenschaftlichen Erkenntnisse. Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL), Braunschweig: 9-15

PISTORIUS, J., T. BROBYN, P. CAMPBELL, R. FORSTER, J.A. LORSCH, F. MAROLLEAU, Ch. MAUS, J. LÜCKMANN, H. SUZUKI, K. WALLNER & R. BECKER (in press): Assessment of risks to honey bees posed by guttation. – Julius-Kühn-Archiv

ROGERS, R.E.L., & J.R. KEMP (2004): Assessing Bee Health in the Maritimes: A survey of pesticide residues in honey bee, *Apis mellifera*, colonies. Final Report, PEI Adapt Council Project Number 319.02. October 15th, 2004

SCHMUCK, R., R. SCHÖNING & R. SUR (2005): Studies on the Effects of Plant Protection Products Containing Imidacloprid on the Honeybee, *Apis mellifera* L. – In: FORSTER, R., E. BODE & D. BRASSE (eds.): Das "Bienensterben" im Winter 2002/2003 in Deutschland – Zum Stand der wissenschaftlichen Erkenntnisse. Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL), Braunschweig: 68-92

SOROKER, V., A. HETZRONI, B. YACOBSON, H. VOET, S. SLABEZKI, H. EFRAT, & N. CHEJANOVSKY (2009): Colony losses in Israel: incidence of viral infection and beehive populations. - In: Proceedings of the 41st Apimondia Congress, Mointpellier, France: 38

VAN ENGELSDORP, D., J.D. EVANS, C. SAEGERMAN, Ch. MULLINS, E. HAUBRUGE, B.K. NGUYEN, M. FRAZIER, J. FRAZIER, D. COX-FOSTER, Y. CHEN, R. UNDERWOOD, D. TARPY & J.S. PETTIS (2009): Colony Collapse Disorder: A Descriptive Study. – PLoS ONE 4: doi:10.1371/journal.pone.0006481

VAN ENGELSDORP, D. & M.D. MEIXNER (2010): A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. - Journal of Invertebrate Pathology **103**: S80–S95

VAN ENGLESDORP, D., N. SPEYBROECK, J.D. EVANS, B.K. NGUYEN, Ch. MULLINS, M. FRAZIER, D. COX-FOSTER, Y. CHEN, D. TARPY, E. HAUBRUGE, J.S. PETTIS & C. SAEGERMAN (2010a): Weighing Risk Factors

Associated with Bee Colony Collapse Disorder by Classification and Regression Tree Analysis. - Journal of Economic Entomology 103:1517-1523.

VAN ENGELSDORP, D., J. HAYES Jr., R.M. UNDERWOOD & J.S. PETTIS (2010b): A survey of honey bee colony losses in the United States, fall 2008 to spring 2009. - Journal of Apicultural Research **49**: 7-14

UNDERWOOD, R.M. & D. VAN ENGELSDORP (2007): Colony Collapse Disorder: Have We Seen This Before? - Bee Culture **35**:13–8

5.1.2.e, 2012-04-10