A critical appraisal of the German Bee Monitoring Project*

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Final

* This is the updated version of the original critique in German

"Das Deutsche Bienenmonitoring-Projekt: Anspruch und Wirklichkeit. Eine kritische Bewertung"

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ABSTRACT. The German Bee Monitoring Project (DeBiMo) is an observational, longitudinal, multi-center study which ostensibly aims to investigate the various factors involved mass bee-deaths in Germany. The study started in 2004 and is ongoing. Results for 2004-2008 were published by Genersch et al (2010). What follows is a critical review of this publication.

DeBiMo is directed by a project board including members of nine German Bee Institutes, two apiarist associations (DIB and DBIB), the German Farmers`Federation (DBV), the Federal Ministry of Nutrition, Agriculture and Consumer Protection (BMELV), and industry (BASF SE, BayerCropScience AG, Bayer HealthCare AG, and Syngenta). Until 2010, the pesticide industry provided 50% of the funding; as of 2010 the project is totally funded by BMELV.

About 110 German experienced beekeepers were asked to participate and they provided 10 colonies each for monitoring during the study. Regional distribution appears to be biased towards areas with less intensive agriculture, where pesticide-use is low. Overall, winter colony-loss among the study-hives ranged from 3 to 16% compared with the estimated norm of about 30% for all of Germany. For these reasons, the study cannot be considered as representative of all German beekeepers, nor of the true scale of bee-colony losses in Germany.

Data collection was incomplete and inconsistent. While beekeeping conditions and varroa samples were collected with relatively few gaps, the data collection on Nosema and virus infestation and chemical residues shows serious gaps. Data collection for chemical residue analysis in bee bread was performed in about 5% of all bee hives.

The statistical evaluation of results is inadequate. In particular, the authors avoided any multi-variate approach to clarify the respective contributions of crucial factors such as pesticide-exposure, varroa, and viral pathogens on winter death of colonies. Inappropriate statistical methods are used to twist evaluations suggesting significance close to proof of causality.

The authors claim that Varroa is "*the dominant killer of honey bee colonies during winter"*. However, the relationship between autumn Varroa infestation and winter-mortality is depicted by a misleading graph, without legend, that obscures the incertainty of relationship.

The main flaw: The authors are either unaware of the most basic principles of epidemiology or appear to be deliberately confusing statistical association for causality in order to reach preconceived conclusions not supported by the underlying data or the methods used to collect them.

Bee-pathogenic viruses were investigated qualitatively. The presence of Deformed Wing Virus (DWV) and Acute Bee Paralysis Virus (ABPV) was significantly associated with winter mortality. The authors misinterpret these observations as *effects* rather than statistical *associations*, resulting in the unsubstantiated claim that "pests and pathogens" (Varroa and viruses) are the main causes of winter losses.

All aspects of the study which related to pesticides were handled superficially. In particular, with few exceptions, no numerical results for pesticides in bee bread were reported. Moreover, the pesticide-profiles (names and concentrations of pesticides in bee bread and pesticide-treated crops in the vicinity) of individual monitoring apiaries were not revealed. Assessment of potential negative effects was based solely on LD50 values, whereas synergistic and sublethal or chronic effects of pesticides were not discussed. This creates the impression that the study was designed to exclude pesticides from the picture and to exonerate the systemic neonicotinoids from all suspicion of involvement in the bee colony losses.

Several points suggest a conflict of interest within the project board. These include the number of non-scientists appointed to the board, the existence of a secret “working group on plant protection” within the board which is in charge of all aspects relating to pesticide analyses and reporting, delay in pesticide analyses, failure to report analytical results of pesticide analyses, the undefined plausibility check of data, and omission of data. Moreover, the publication (in Apidologie) does not reveil who was responsible for designing the study, for writing of the manuscript and for the final version of the manuscript. Last but not least, it is remarkable that the 3 reviewers of the DeBiMo publication failed to detect the main flaw and the numerous deficiencies.

Taken together, it is clear that the study does not accord with ‘the scientific spirit’. For these reasons, DeBiMo has failed to deliver a thorough and credible scientific evaluation of the possible causes of bee-colony death in Germany. It has failed to prove that Varroa and viruses (“pests and pathogens”) are the primary causes and that “pesticides play no role”.

The following is an appraisal of the publication by Genersch et al (1) who report on a 4-year period (2004 – 2008) of the German Bee Monitoring Project (**De**utsches **Bi**enen **Mo**nitoring, DeBiMo). Anonymous interim reports appeared for the years 2004 -2008 (2) and 2008-2009 (3), annual results for 2010 and 2011 were reported by Rosenkranz et al. (4).

DeBiMo has been described by the authors, members of the project board, and the media as a globally unique study that aims to investigate and solve the enigma of high honeybee colony losses in Germany. Currently the authors state a less ambitious aim, viz: "to generate important data on the course of bee losses". By and large, the Federal BMELV, the State Ministries of Agriculture, and most of the media, have accepted the conclusions of DeBiMo as 'authoritative'. COLOSS, a huge follow-up study initiated by the EU, allegedly used DeBiMo as a blueprint for its own work.

At the time of writing this critique, bee losses are skyrocketing in the US: according to Jeff Pettis, research leader at the Federal Agriculture Dept of the US, "last winter`s death rate is much higher than it`s ever been" (32). Mr. Adee, the largest beekeeper of the US, had a 55 percent loss, and Mr. Dahle lost 10,000 hives of bees until February 2013, a 77 percent loss (32). For these reasons, a critical evaluation of the DeBiMo publication (Genersch et al (1)) is urgently required.

At the outset of DeBiMo it was generally assumed that colony losses are caused by a variety of factors acting together. These include the ecto-parasitic mite Varroa destructor, environmental stress, transgenic crops, lack of food variety, a toxic ingredient (hydroxyfurfural) in high-fructose corn syrup which is commonly fed to bees in the USA, electromagnetic radiation from cellphone towers, antimicrobial and acaricidal chemicals used by beekeepers to control infectious diseases and Varroa mites, and the widely used agrochemicals including the neonicotinoid insecticides.

Neonicotinoids are widely accused of being the main if not primary cause of sudden colony death, recently designated as Colony Collapse Disorder (CCD) in the USA. It was the mounting scientific evidence for a major role of neonicotinoids in bee mortality that appeared to have motivated Bayer, BASF and Syngenta to contribute to the funding of and thereby enabling the start of DeBiMo.

French bee keepers reported that Bayer's Gaucho®, containing the neonicotinoid imidacloprid, a seed treatment for corn, oilseed rape and sunflower had killed over one million colonies from 1994-97. As a result, in 1998, the French Minister of Agriculture M. Glavany, ordered a Comite Scientifique et Technique (CST) of independent scientists from the French National Center for Scientific Research (CNRS) to conduct a "multifactorial investigation of the problems in bees".

The resulting expert assessment (CST report, 5) is by far the most comprehensive scholarly investigation of the effects of imidacloprid on honey bees. It concluded that "the risks (of imidacloprid) to bees are of great concern and that findings agree with reality, viz. high losses of forager bees, high winter-colony-losses, and the prevalence of behavioural problems in areas dominated by sunflowers and corn". It also pointed out that sublethal doses of imidacloprid increase the bees' susceptibility to natural diseases and parasites. As a result, in 2000, the French government imposed a ban on the use of imidacloprid for seed-treatment of sunflowers, oilseed rape and corn, which remains in force to this day.

It is telling that the DEBIMO authors make no reference whatever to the expert assessment of the French Comite Scientifique et Technique, which represents the highest level of scientific inquiry that the French State ever convenes.

When we asked, why the CST assessment was not cited, we were told "because it represents the personal opinions of the authors and because it was not published in a peer-reviewed scientific

journal like Apidologie". What this excuse fails to mention is that the CST considered over 120 peer-reviewed studies and eye witness statements from all over Europe; it remains the most comprehensive review of the published scientific evidence on imidacloprid yet undertaken.

Monitoring Studies versus Controlled Experiments

A 'monitoring study' sets out to observe and record an outcome, over a certain period of time, in a systematic way. The aim of a monitoring-study is to develop hypotheses about which factors appear to affect the outcome. However, it must be understood that a monitoring-study is wholly unsuitable for the purpose of proving or disproving a causal relationship. The only way to prove or disprove a cause-and-effect-relationship is through controlled (hypothesis-driven) experiments, where treated and untreated (=control) groups are compared. This crucial difference between a 'monitoring study' and a 'controlled experiment' is often misunderstood, even by experienced scientists.

DeBiMo is a multi-center, longitudinal study. Two preconditions are essential for appropriate conduct of studies and experiments: establishment and approval of a study protocol by the investigators before starting the study, and quality control by an independent monitor. Multi-center studies in particular require the participation of a study monitor, a scientific 'watchdog' who aims to guarantee that the study protocol is adhered to at all centers and at all times. DEBIMO did not have a study monitor. It was even started without a pre-approved study protocol. This resulted in inconsistencies of data collection, and temporary data-chaos.

DeBiMo records a number of factors which may affect the survival of bee-colonies over the winter, these include:

- Type of beehive used (wood, polystyrene, insulated etc.)
- Maintenance and management methods (winter-feeding, Varroa treatment etc.)
- Colony-strength in autumn and spring,
- Age of queen,
- Level of infestation by Varroa mites
- Prevalence of Nosema and five viruses (DWV, KBV, ABPV, SBV, and IAPV) ,
- Pesticide residue analysis in honey and bee-bread (stored pollen)

Data on mite-control measures, on exposure to certain crops, and on exposure to oil-seed rape was also collected, but was not presented. Results were only reported for 2004 to 2007; no explanation was given as to why data for 2008 is missing.

DEBIMO is not representative of beekeeping in Germany

The first steps in designing a monitoring study are to determine an adequate sample-size (number of participating beekeepers and hives) and the selection method for the bee keepers. This is important to ensure a balanced representation of all relevant factors of influence and to provide sufficient statistical power. These crucial steps were neglected.

About 110+ "experienced" beekeepers from most of the German Federal States were *asked to participate*. No inclusion and exclusion criteria, used for the selection of beekeepers, were given. In fact, most of the selected beekeepers were previous collaborators with the Bee Institutes. Such an obvious lack of 'randomization' predisposes any study to 'selection bias'. Assuming that there are 82,000 German beekeepers who manage about 900,000 beehives, the monitoring-study selection represented less than 0.15% of all beekeepers and just 0.5% of all beehives. Moreover, although the study tried to select a cohort of beekeepers that is representative of the wide range of environmental conditions and regions typical of beekeeping in Germany, this was not entirely accomplished. For instance, according to Figure 1, no beekeepers at all were included from Saarland or Schleswig-Holstein. Moreover, beekeepers who were under-represented include those from: Schleswig-

Holstein, Lower Saxonia, Mecklenburg-Vorpommern, and northern Bavaria. These under-represented areas are characterized by highly intensive farming, dominated by pesticide-treated crops. In contrast, areas with less intensive agriculture (mountains, forests, heaths etc.) appear to be over-represented in the study.

Data collection and processing is questionable and authorship doubtful

Each participating beekeeper selected ten of his colonies for the monitoring-study. This arbitrary personal-choice could have been influenced by a number of factors like: ease of handling, historical viability, convenience in management etc. In order to avoid such possible bias, the monitoring colonies should have been selected by a strict randomization process.

Winter-death of monitored colonies was not compared with all of the hives from the entire apiary, an obvious and simple approach to cross-validate the colony losses. This contravenes the study-protocol which stipulates that winter losses should be recorded for the entire apiary.

Only complete data sets were considered for statistical analysis and these had to pass a plausibility check. No details are given as to how the plausibility check was done. In view of the range of stakeholders on the project board, a detailed explanation was imperative, so as to rule out any suspicion of bias in data-collection and processing. Since no detailed explanation is given, data selection and statistical analysis are not transparent and do suggest bias.
For evaluation 17% of the data sets (885 of 5,198) were dropped on account of missing information. Compared to all 5,198 datasets on the level of bee hives, 24% of varroa data, 59% of Nosema infestation data, 67% of virus infestation (DWV/ABPV/SBV) and 95% of all chemical residue samples were missing or not reported. Nosema and virus infestation as well as residue analysis data were not collected systematically and consistently over the years of observation to cover all beehives under study. This rules out the possibility to analyze these data in a multi-factorial model , and no attempt was made to try this as far as possible.

Publication of results and presentations are coordinated by the project board. Authors publishing in *Apidologie* are not obliged to declare conflicts of interest. Nor are they required to declare who was responsible for:

- Designing the study,
- Statistical analysis,
- Writing the manuscript
- Writing the final draft.

According to Genersch et al (2010) the responsibilities of the Scientific Bee Institutes included: “the coordination of the field work, the data collection, and the supervision of the beekeepers involved”. This leaves room for speculation as to, who had the leading role in data analysis, who wrote the manuscript and who wrote the final draft?

DeBiMo provides no proof that *Varroa destructor* is the main culprit

The mean percentage for Varroa infestation (mites/100 bees) in autumn 2004 to 2007 was 3.4 in surviving colonies as opposed to 15.1 in colonies that did not survive winter. Winter -losses were not randomly distributed but clustered. It is evident from Figure 4 that the majority of beekeepers suffered no losses, whereas about 5% of beekeepers suffered losses of 90 to 100% of their hives. In addition, regional differences in losses were not consistent over the 4 years and not consistently related to certain beekeepers. Clusters indicate ‘confounders’, e.g. some unidentified factor(s) that affect(s) the outcome, occasionally or irregularly. Clusters should be taken as an incentive to look for and possibly identify such confounder(s), but there are no indications that this was taken into

consideration. For instance, no hypothetical confounders were considered or discussed to help explain why 5 % of the monitoring beekeepers lost 90 to 100 percent of their monitoring colonies.

Figure 5 presents the relationship between Varroa infestation rate in autumn and winter-mortality. It gives the impression that winter mortality and Varroa infestation rate are closely related; an impression that is underlined by the high Spearman rank correlation coefficient of r = 0.996. The plot is based on a mere 14 points, and the legend does not explain what the points denote. Upon inquiring how the graph was made, we were told that it was based on a total of 3,589 colonies, where colonies with the same rate of infestation were divided into "infestation groups" (zero Varroa/100 bees, 1-2 Varroa/ 100 bees asf) and the mean percentage mortality of each infestation group was used as the data point (H. Kaatz, pers. communication). **Graphical representation of Figure 5 is inappropriate and misleading as it intentionally omits the wide dispersion of data.** In consequence, the correlation coefficient is grossly overestimated, suggesting a much closer correlation than would result from an unbiased estimate based on a logistic regression model using all 3,589 observations instead of just 14 means. Inclusion of all observations (as a scatter graph or as a box plot, methods these authors had used in earlier publications) would provide a true picture. A scatter graph would likely show that some colonies collapsed despite having very few Varroa, or indeed, no Varroa at all. In fact, upon asking to see the data, we were shown that in the lowest "infestation group" (0 Varroa/100 bees) some 23 colonies died during winter (H. Kaatz, personal communication). Likewise, a scatter graph would reveal that some highly-infested colonies did in fact survive the winter, an observation that has been reported earlier. Regrettably we were unable to reanalyze the data set as the authors refused to release the raw data.

The authors repeatedly claim that Varroa mite infestation was *"undoubtedly the main cause of overwintering problems"* and again, in no uncertain terms, *"based on the results presented (!) it is safe to state that Varroa destructor is the dominant killer of honey bee colonies during winter"*. **This assertion is false, because it equates association with causation.** Notably, the interim reports (2,3) stated correctly, that Varroa and winter mortality showed a highly significant correlation. We conclude that exchanging *correlation* (Interim Reports) for *cause* (Genersch et al, 2010) indicates the intention to mislead.

In a contemporary publication by W. Ritter, coauthor of Genersch et al. (2010), the following statement is found: *"*Like all descriptive studies, definite statements cannot be made concerning factors causing CCD, and there is no clear evidence to date to suggest that Varroa is, or is not, involved (13)*"*. This is a direct contradiction and rebuttal of the main conclusion of the German monitoring study.

To corroborate their (unsubstantiated) claim that Varroa is the main cause of mortality in winter, the authors (1) refer to studies in the US and Europe, which purportedly prove Varroa to be a "main factor for winter losses in the US and Europe". These quotes from the literature are incorrect. In fact, the studies in the US, which compared apiaries affected by CCD with unaffected apiaries, found no difference in the percentage of colonies infested with Varroa, nor in abundance of Varroa (18, 21). Nor does the descriptive pathology study of bee samples from Poland provide any evidence that Varroa was the cause of colony death. (20).

The speculative role of pathogens

Bee samples were analyzed for the presence of Nosema sp. and viruses, acute bee paralysis virus [ABPV], sacbrood virus [SBV], deformed wing virus [DWV], Kashmir bee virus [KBV], and Israeli acute paralysis virus [IAPV]). The methodology of virus detection is described comprehensively. Highly significant associations were claimed for the presence of two viruses, viz. DWV and ABPV, with winter mortality. The heading of Table 5 (*Effects* of pathogen …) is incorrect and misleading because, as noted above, the results indicate associations, not effects.

Likewise, the claim that DWV infection (based on virus detection in the head) in autumn has a highly significant negative effect on winter mortality, is incorrect. DWV is highly prevalent in honeybee colonies but has low virulence, particularly in the absence of Varroa (17). Thus, DWV is merely one factor, among many, that may contribute to colony losses; but this study provided no evidence that it did contribute. This is in line with the authors' citation that: "unfortunately, DWV incidence of 90-100% in all colonies regardless of whether they are strong, weak or collapsing does not allow correlating DWV infection with colony losses since the mere presence of DWV in otherwise healthy bees is obviously of no clinical relevance." It is also claimed that there was no *negative effect* of SBV or KBV on winter survival, once again confusing association for effect. This claim is misleading.

The factors involved in causing winter losses were enumerated as "high mite infestation levels, clinically relevant DWV and ABPV infection in autumn, old queens, and colony weakness in autumn". The purported role of Varroa and viruses ("pests and pathogens") as the prominent causes of colony losses has been reiterated by others (29) in spite of the lack of convincing evidence.

The relevance of pathogen analyses for explaining winter losses is dubious. Firstly, correlations do not prove causation. The mere presence of pathogens does not reveal anything about clinical relevance. Secondly, the authors use varying terms (prevalence, incidence, infection, clinically relevant infection, negative effect, association, relationship) and verbal restrictions (involved, contribute, implicated) that qualify their assertions. This weakens the claim that pathogens are causes of bee mortality.

Pesticides are investigated superficially

All aspects related to pesticides were highly controversial from the beginning of the study. In 2006, the German Professional Beekeepers' Association (DBIB) protested in an open letter, that no serious efforts were being made to sample and analyze bee-bread for pesticides. The Association threatened to resign from the project board unless steps were taken to take the pesticide issue seriously (17).

All matters related to pesticides, viz. sampling, analyses, interpretation and reporting of results were handled by a secret "working group of plant protection agents" within the project board. Members included, *inter alia,* four employees of BayerCropScience, BASF and Syngenta, as well as the analyst in charge of pesticide analysis.

Pesticide analyses in bee-bread from 2005 and 2006 were carried out by Bayer CropScience, but this was not communicated. Later on this work was taken over by a chartered laboratory (LUFA Speyer). The multi-compound method used by LUFA Speyer has relatively low sensitivity. For example, the limit of detection for all neonicotinoids was 1 microgram/kg, compared with 0.1 microgram/kg (for the neonicotinoid imidacloprid) as described by Bonmatin et al.(25). Surprisingly, the analyst of LUFA Speyer is not among the authors.

Sampling for residues analysis was done in only 5% of bee hives. Just one sample from one frame in one bee hive was used to represent the 10 hives of each bee keeper. At the level of bee keepers, 54% were not sampled for bee bread. We doubt that this sampling method is appropriate and sufficient to investigate the role of agrochemicals. For these reasons, the results of residue analyses need to be interpreted with caution.

Figure 6 depicts the percentage of rape-pollen found in honey harvested in summer 2006 correlated with the overwintering-ratio. The latter was determined by an unvalidated method by counting the number of "combs covered by bees" and by dividing the number in Spring by the number in

Autumn, an unvalidated method, rather than the validated Liebefeld Method. Figure 6 is puzzling because 142 bulk samples of honey were collected, yet the graph shows only 79 data points, omitting 44% of the total samples. Based on Figure 6 the authors conclude that *"the hypothesis could not be verified that intensive contact of honey bee colonies with oilseed rape has a negative influence on overwintering".* This conclusion is objectionable because 1., a monitoring study is generally not suited to prove (verify) or disprove an effect. 2., the data set is grossly incomplete, and 3., about nine data points (of 79) show an overwintering coefficient greater than unity that would indicate an *increase* of colony strength in winter, which is close to impossible for individual colonies much less for an entire apiary of 10 colonies represented by each data point. These data likely result from the unvalidated method for estimating colony strength and from poor control of the timing of the estimation. "To avoid overestimating the population size of the overwintered colony the population estimation had to be performed prior to the emergence of the first spring brood. Therefore, the last accepted period for measuring the starting population was the 15th week of the year". Given that the time of emergence of the first spring brood varies markedly with the region, the seeming increase of colony strength in winter is most likely due to the estimation taking place too late.

In response to our critique the authors replied that the missing data were due to "overlapping of data points". In a later response they admitted that they had included only data points originating from a narrow time window of sampling. Sub-sampling is acceptable, providing that reasons are explained and sound. As this is lacking, additionally to the lack of validity of the data given above, the interpretation of Figure 6 is doubtful. Omission of data without good reason and explanation is commonly interpreted as an indicator for the possibility of fraud in science (30).

A total of 56 pesticides were detected in bee-bread, comprising: 18 insecticides, 27 fungicides, and 11 herbicides (Table 7). **Only the names and prevalence of these pesticides are given but no concentrations are reported**. As the pivotal point of a quantitative analysis is the concentration, *w*e view this as a smokescreen and a mockery of beekeepers`concerns. Table 7 even falls short of the meagre results reported in the Interim Reports (2,3,4) where, for each pesticide, at least the frequency of values "above limit of quantitation" and "below limit of quantitation" are reported, but no actual concentrations. As pesticide concentrations and prevalence in bee bread have been reported in several papers (see for instance, Ref.10), Table 7 adds very little to present knowledge. The withholding of analyzed pesticide concentrations makes it impossible to judge potentially toxic effects and precludes comparisons with other authors` work. A state-of-the-art publication on pesticide residues in bee products in the excellent US study detected a total of 98 pesticides and metabolites (12).

Upon our request LUFA Speyer declared that analytical results of pesticide analyses are as a matter of course reported as numerical concentrations. The fact that these were deliberately withheld in the publication indicates a conflict of interests in the project board.

As for insecticides in bee-bread, the neonicotinoid thiacloprid was most prevalent (9 positive samples, maximum level 199 µg/kg) with increasing prevalence from 8 % of samples in 2005+2006 to 51% in 2011 (5), followed by dimethoate (organophospate), azetamiprid (neonicotinoid), pirimicarb (carbamate), tau-fluvalinate (pyrethroid for varroa control), and lambda-cyhalothrin (pyrethroid). With the inclusion of coumaphos (organophosphate, varroacide) this amounts to a total of 7 neurotoxic compounds.

The most abundant drugs and pesticides were coumaphos, boscalid (fungicide) and terbuthylazine (herbicide). Some samples were stated to contain "quite high" residue "amounts", an unacceptably vague statement. However, readers are reassured that "these residue amounts did not correlate with

poor colony development". One wonders how the correlation was determined and why the actual values used for the calculation were kept secret.

Very recently, Palmer et al (2013) demonstrated that coumaphos and the neonicotinoids imidacloprid and clothianidin share the same neurophysiological mode of action. The resulting impairment of learning and behaviour is observed at concentrations that are encountered by foraging bees and within the hive, and they are additive with combined application.

Based on bee-bread analysis in 2006, the authors claim that no significant difference was found in the overwintering ratio between apiaries with no pesticide residues and those with > 10 µg/kg of at least one active ingredient. The validity of this claim is doubtful for several reasons:

1. The threshold value of 10 µg/kg is arbitrary as it is based on acute toxicity (LD50) which varies widely between pesticides,

2. The number of analyses (105 samples from > 4,000 colonies) was low and hence, the statistical power was not sufficient to detect a difference.

3. Sublethal cumulative effects which are elicited by very low concentrations (15) are disregarded and

4. Synergistic effects of pesticides are not taken into account.

Sublethal effects are effects elicited by very low concentrations of insecticides that are typically found in nectar and pollen of crops (5). They appear to affect insect health more widely than the obvious cases of acute intoxication. On account of their neurotoxic effects sublethal doses of neonicotinoids impair virtually all cognitive, memory, learning, other behavioral and locomotor functions, most obviously the homing-ability of honeybees, which can result in colony losses (5). Suchail et al. reported that the cumulative dose of imidacloprid required to elicit sublethal effects was 60 to 6,000-fold lower than the acutely toxic dose (15). Neonicotinoids bind almost irreversibly to receptors in the nervous system and accumulate over time (20). Sub lethal concentrations in nectar and pollen are so infinitesimally low that they often escape detection by even the most technologically advanced lab techniques.

Synergistic effects can accrue from combined-application of certain pesticides as a 'cocktail mix', a common practice in agriculture. As opposed to an additive effect, synergism results in potentiation (multiplication) of the (ill-)effect. Because honeybees have limited xenobiotic detoxification enzyme (P450) activity they are particularly vulnerable to pesticides (8). As an example, when the DMI-fungicides ("Azols") triflumizol and propiconazole were applied together with thiacloprid, the oral toxicity of thiacloprid increased 1,124-fold and 559-fold, respectively (5). For the additive effects of Coumaphos and neonicotinoids, see above (33).

An increase in toxicity by synergy was also found when DMI-fungicides and pyrethroids (insecticides) were applied together (23). Likewise, synergism between the intestinal pathogen Nosema and imidacloprid significantly increased the toxicity of imidacloprid and weakened honeybees (7). Such synergy may also occur when Varroa mites and pesticides combine to affect bees. Because of the vast variety of pesticides in the marketplace, the common co-application of pesticides, and ever-increasing acreage of use, it is highly probable that many potentially synergistic combinations are as yet unknown or remain unreported in the literature.

DeBiMo does not reveil the pesticide-profiles (which pesticides were found at what concentrations) of individual monitoring apiaries. As six DMI-fungicides and 3 neonicotinoids

(imidacloprid, thiacloprid and acetamiprid) were detected in bee bread there is a possibility that synergistic toxic effects were present in certain apiaries but went unnoticed or were not reported.

No efforts were made by DeBiMo to consider the potential risk of sublethal and synergistic effects of pesticides. Evaluating the pesticide analyses in bee-bread and the pesticide application protocol of farms within flying range of monitored colonies may have provided hints of exposure.

In order to build hypotheses, monitoring studies require an inquisitive mind, similar to that typical of a criminal investigation. There are no indications that this attitude prevailed in DeBiMo. As a rule researchers tend to recoil from any association with pesticide research (26).

No mention was made of the accidental intoxication of honey bees by clothianidin via treated maize seed, which severely affected over 20,000 German bee colonies in 2008 (7). Even though no DeBiMo apiary was affected (H.Kaatz, personal communication), an independent monitoring study should also record relevant events *outside* the monitoring.

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