Woodcock, Ben A.; Heard, Matthew S.; Jitlal, Mark S.; Rundlof, Maj; Bullock, James M.; Shore, Richard F.; Pywell, Richard F.. 2016. **Replication, effect sizes and identifying the biological impacts of pesticides on bees under field conditions.** *Journal of Applied Ecology*, 53 (5). 1358-1362. 10.1111/1365-2664.12676

Replication, effect sizes and identifying the biological impacts of pesticides on bees under field conditions.

Woodcock, B.A.[1], Heard, M.S. [1], Jitlal, M.S. [1], Rundlöf, M.[2], Bullock, J.M. [1] Shore, R.F.[3] & Pywell, R.F.[1]

1. NERC Centre for Ecology & Hydrology, Maclean Building, Crowmarsh Gifford, Wallingford, Oxfordshire OX10 8BB, UK. Tel. +44(0)1491692415

2. Lund University, Department of Biology, 223 62 Lund, Sweden.

3. NERC Centre for Ecology & Hydrology, Library Avenue, Lancaster Environment Centre, Lancaster, Bailrigg LA1 4AP

Journal of Applied Ecology: Scientific Forum Article

Word Count: 3973

Running title: Field-based evaluation of bees

Summary (150 words)

1. Honeybees have worldwide importance as crop pollinators. To ensure their persistence in agricultural systems statistically robust field trials of plant protection products are vital.
2. We consider the implications of regulations from the European Food Safety Authority that require the detection of a 7 % effect size change in bee colony sizes under field conditions.
3. Based on a power analysis, we argue that the necessary levels of replication (68 replicates) may pose practical constraints to field testing.
4. *Synthesis and applications*: Regulatory studies benefit from data sources collated over a range of spatial scales, from laboratory to landscapes. Basing effect size thresholds solely on expert judgement, as has been done, may be inappropriate. Rather definition through experimental or simulation studies that assess the biological consequences of changes in colony size for bee populations is required. This has implications for regulatory bodies outside the European Union.

Key words: bumblebees, experimental design, honeybees, neonicotinoids, pollinators, statistical power testing, regulatory risk assessment.

Introduction

The agricultural sector relies heavily on chemical pesticides to protect crops from a wide range of pests (Tilman *et al.* 2001; Oerke 2006). The safe use of these pesticides depends upon robust and effective risk assessments that balance the need to support food production while protecting the environment and supporting ecosystem processes (EFSA 2013). As

50 domesticated and wild bees have high risks of exposure to pesticides in agricultural systems,
51 regulatory risk assessments protect both their biodiversity and contribution to crop production
52 through pollination (Gallai *et al.* 2009; Potts *et al.* 2010; EFSA 2013; Vanbergen *et al.* 2013).
53 If the evidence provided by these risk assessments is to be robust then experimental designs
54 need to reduce to agreed acceptable levels the likelihood of failing to reject a false null
55 hypothesis, specifically a Type II statistical error whereby a real effect of a pesticide on a bee
56 population is not detected due to insufficient experimental replication (EFSA 2013). The
57 more variable systems are, or the smaller the effect sizes (the difference between pesticide
58 and control treatments) to be detected, then the greater will be the need for replication to
59 detect these differences reliably (Cresswell 2011; EFSA 2013). This Policy Directions paper
60 aims to examine the practical implications associated with European Food Safety Authority's
61 (EFSA) guidance on addressing this issue and the implications that it has for future field and
62 landscape scale evaluations of pesticide impacts on bees.

63 The regulatory basis of pesticide risk assessments for bees have developed over many
64 years from *ad hoc* combinations of laboratory, semi-field (e.g. tented colonies such as those
65 described in Colin *et al.* 2004) and field-based evaluations (EFSA 2013; Medrzycki *et al.*
66 2013). These studies aimed to identify the consequences of a wide variety of mechanisms of
67 exposure to pesticides, including direct contact, consumption (pollen, nectar and in water)
68 and impacts from pesticide metabolites (EFSA 2013). Laboratory assessments of acute oral
69 and contact toxicity (e.g. LD_{50} tests) have historically represented the cornerstone of this
70 process, and are based on well-established fixed protocols developed by regulatory bodies
71 (OECD 1998a, b; EPPO 2010; CEB 2011). For example, LD_{50} mortality protocols require the
72 use of a control (e.g. untreated sugar), a known toxic reference and a test compound applied
73 at five doses; each replicated at least three times (OECD 1998a, b; EPPO 2010; CEB 2011).
74 Although such experiments provide robust estimates of pesticide toxicity, their focus has

been on acute mortality effects of individual bees identified over short time periods, e.g. 48 hours. These assessments do not take into account additive effects that may result from chronic sub-lethal impacts of pesticide exposure over extended periods of time on colonies (Cresswell 2011; EFSA 2013) or the potential effects of exposure to multiple pesticides (e.g. Gill, Ramos-Rodriguez & Raine 2012; Johnson *et al.* 2013; Williamson & Wright 2013). Over the last decade risk assessment practices within the European Union have been widened to include assessments of not only acute oral and contact toxicity on individual worker bees (e.g. OECD 1998a, b), but also assessments of colony level impacts resulting from repeated or chronic exposure (EFSA 2013; Medrzycki *et al.* 2013).

A more recent requirement has been the use of field-based studies that allow more realistic behavior of bees to be considered (EFSA 2013). This is especially important for eusocial species that have potentially large foraging ranges and so are capable of utilizing spatially complex foraging resources over large areas (Mommaerts *et al.* 2010; Potts *et al.* 2010; Kennedy *et al.* 2013; Vanbergen *et al.* 2013). For such colony-level processes these field-based studies have been crucial for quantifying the impacts of pesticides on population viability, pollination services and yield of hive products like honey (EFSA 2013). While standardised laboratory conditions make regulatory testing tractable, field-based studies are far more susceptible to the inherent variability across both space and time found within real-world systems. Even for domesticated species (e.g. honeybees), replicate colonies can show dramatic differences in growth and survival under almost identical conditions (Cresswell 2011; Pilling *et al.* 2013; Godfray *et al.* 2014; Godfray *et al.* 2015; Lundin *et al.* 2015). Using a systems model approach, Bryden *et al.* (2013) demonstrated that in the case of bumblebees sub-lethal stress (linked to factors like neonicotinoid pesticide exposure) may be the underlying drivers that variability in colony success. Unfortunately, this variability represents a potentially serious problem with regulatory testing. Cresswell (2011) found that

of four field or semi-field studies investigating impacts of neonicotinoid pesticides on honeybees, only one had sufficient replication to detect changes in honeybee performance of less than 33%. Using already known measures of variability from previous studies this problem can be addressed by the use of *a priori* power analyses to predict the experimental replication necessary to detect a specified effect size between control and pesticide treatments. This represents not only a quantitative way of determining the feasibility of a field experiment, but is also a regulatory requirement used to ensure conclusions underpinning regulatory decisions are statically robust (EFSA 2013).

Statistical power for field-based experiments in the EU

To address this problem EFSA have stipulated that field-based studies investigating the impacts of pesticides on bees must have sufficient replication to detect a 7% change in colony size in response to pesticide exposure with a fixed 80% probability (often referred to as statistical power, 1-β) and a significance level of α=0.05 (EFSA 2013). Note that for bee mortality, detection of larger effect sizes are deemed acceptable (Khoury, Myerscough & Barron 2011; EFSA 2013). While field studies provide crucial information about the responses of bees under biologically realistic conditions their resource intensive nature has meant that they are not typically a standard requirement in regulatory risk assessments (EFSA 2013). However, principals for their implementation are laid out in the recent regulatory framework given by EFSA (EFSA 2013).

Using expert opinion ESFA have argued that a 30% reduction in colony size (termed a ‘large’ effect size) would result in a loss of honeybee colony viability, while a less than 7 % reduction (described as a ‘small’ to ‘negligible’ change in colony size) would have no effect (EFSA 2013). Although these assessments were based on honeybees, it is worth considering

their relevance to bumblebees. Whitehorn *et al.* (2012) suggested that for *Bombus terrestris* thresholds in colony sizes are likely below which queen production (the key predictor of reproductive potential) will not occur. This potentially non-linear relationship would make it hard to predict the impact of a 7 % decrease in colony size and so the relevance of this threshold for bumblebees is probably not the same. However, the detection of this 7 % effect size currently represents the minimum threshold for detecting population level changes in regulatory field studies for honeybees (EFSA 2013).

Under controlled laboratory conditions the reliable detection of 7 % effect sizes on bees would be likely to be more feasible as much of the inherent variability of natural systems is removed. However, in the context of field-based studies on honeybees (or other model bee systems like *B. terrestris*) such a detection goal represents a major challenge due to the high levels of replication required to counter between site and inter-colony variability (Cresswell 2011). To date the practical implications of achieving this regulatory detection goal are only recently being considered (EFSA In press).

Practical considerations and replication in field-scale experiments

Applying the power analysis approach outlined by EFSA (2013) on data from a large-scale field experiment investigating the impact of the neonicotinoid pesticides (clothianidin) on honeybees (Rundlöf *et al.* 2015), we find that 68 replicates of treated and control sites would be required to detect a 7 % change in colony size (Supporting Information Appendix S1). Such a power analyses would ideally be undertaken using data relevant to the regional location of the regulatory study. Further, the Rundlöf et al. (2015) study assess colony size using the widely used visual based Liberfeld approach. More advances computer based methods to estimating colony strength may well reduce estimates of between colony

variability and so the sample size required to detect a 7 % effect size (Wang & Brewer 2013). Independent of these caveats, implementing such a large-scale field experiment with sufficient replication to detect a 7% effects size change would be challenging from a practical perspective. Even using relatively small areas of treated crop (i.e. the 1-2 ha suggested by EFSA 2013) establishing 68 replicate blocks would be complicated where spatial separations of 2-4 km between experimental sites are needed to reduce the probability of cross-contamination by foraging bees (EFSA 2013; Cutler *et al.* 2014; Rundlöf *et al.* 2015). Simply achieving uniform agronomic management across so many spatially separated sites, each operated by different farmers, would also be hard to achieve. In addition, such small areas of treated crop (<2 ha) do not reflect real-world agricultural practices where mass flowering crops are often planted in larger homogeneous blocks (>50 ha). Rundlöf *et al.* (2015) in Sweden used more realistic average field sizes of 8.9 ha; however, even these may be relatively small compared to cropping regimes in many countries. An experiment at this scale would require not only the planting, but also the necessary licensing, to sow over 605 ha (e.g. 8.9 ha × 68 replicates) of treated crop. Crops treated with unlicensed pesticides (i.e. which are being risk-assessed prior to any licensing) may well be unsuitable for incorporation into the food chain and would need to be disposed of in an appropriate manner (HSE 2015). It is also quite possible that the public, NGOs concerned with conservation and regulatory authorities with other remits (e.g. water quality) may also object to testing of unlicensed chemical compounds on this scale. There is precedent for such problems in the case of genetically modified crops where wide scale public resistance to testing was seen in the UK (de Krom, Dessein & Erbout 2014).

As the use of pesticides remains crucial to maintaining crop yields there is also an economic case for questioning the appropriateness of this level of replication (Tilman *et al.* 2001; Oerke 2006). For example, field studies of a comparable scale (60 replicates) have

been undertaken in the past – for the Field-Scale Evaluations of genetically modified crops in the UK (Perry et al. 2003) – but have been criticized as being prohibitively expensive and so unsuitable for being repeated as a matter of routine for other crop protection products (Qi *et al.* 2008). These issues are certainly acknowledged by EFSA who are currently considering the increased use of systems based modelling approaches at the cost of field scale testing for the assessment of impacts on honeybees (EFSA In press). Should field studies be used the likely cost linked with this level of replication are high. This can be seen in an ongoing study (see http://www.ceh.ac.uk/our-science/projects/impacts-neonicotinoids-honeybees) from which we calculate that the replication necessary to detect a 7 % change in bee colony sizes would cost upwards of €10.3 m *p.a.* (assuming costs of €75.7 k per site, see Supporting Material Appendix S2). These costs also assume only a single study year, something that is likely to be less than ideal where long term effects of pesticides may have chronic effects. For example, using a systems model approach Becher *et al.* (2014) demonstrated that changes in honeybee colony sizes following exposure to neonicotinoid pesticides would only be detectable after five years. The use of studies across multiple years, while being biologically more meaningful, would add significantly to the cost of this research (e.g. €51.5 million for a five year study). Given that the development costs of a typical plant protection product are estimated at €40m (McDougall 2010), such an increase to satisfy only one part of a regulatory process may impact the commercial development of some compounds.

Conclusions

If we are to sustainably feed a rapidly growing global population then agriculture will need to become increasingly intensive, while simultaneously limiting its impact on biodiversity (Oerke 2006; Bruce 2010; Godfray *et al.* 2010). The development of a new

198 generation of effective but environmentally safe pesticides represents one of several
199 approaches that may contribute to supporting future crop yields. To assess and minimize
200 risks to the environment, pesticide regulatory frameworks may increasingly need to use
201 information across a range of spatial and temporal scales. Importantly, risk assessment may
202 need to use field trials that determine the long-term impacts (>1 year) of exposure on species
203 and populations. Due to a non-linear relationship between effect size and replication the
204 detection of 15 % and 20 % changes in colony size would require considerably lower levels
205 of replication (respectively 13 and 7 replicate blocks, Fig. 1, Supporting Information
206 Appendix S1). If the detection of alternative effect sizes retain biologically meaningful
207 information about the impacts of plant protection products then such field studies may have
208 an economically viable part to play in the future regulatory framework. While we strongly
209 endorse the need for power analysis, we suggest that a 'one size fits all' effect size of 7 %
210 therefore need to be further justified by informed debate supported by experimental evidence.
211 Considering the case of pesticide impacts on bees we suggest that a more cost-effective and
212 biologically meaningful strategy for regulatory testing would be to follow a process that
213 included the: i) use of experimental and simulation modelling approaches to define
214 biologically meaningful threshold effects for bee population persistence in field experiments;
215 ii) use power testing to determine the level of replication required to identify reliably these
216 lower detection goals; and iii) utilise the savings in resources to examine the impacts of
217 pesticides over a number of years rather than in a single year.

218 Ultimately studies need to be fit for purpose in terms of their ability to detect
219 population changes, while being realistic in terms of practical implementation. Other
220 complementary sources of evidence may also support and inform the regulatory process,
221 further strengthening experimental field assessments of pesticide impacts on bees. For
222 example, well designed and geographically targeted pollinator monitoring schemes (e.g.

223 Defra 2014) could provide early warnings of long-term, sub-lethal impacts of pesticides on a
224 wide range of other wild bee species. Importantly this would extend long term assessments
225 beyond the limited number of species (e.g. *Apis mellifera*, *B. terrestris* and *Osmia bicornis*)
226 currently suitable as model systems. Indeed, analysis of monitoring data has recently
227 provided evidence of negative associations between pesticides and long-term demographic
228 trends on taxa other than bees (e.g. Hallmann *et al.* 2014). Large scale field based
229 assessments are always likely to remain costly and so would only represent a final stage in
230 the regulatory process. However, field scale studies identifying the impact of pesticides
231 provide key validation under real world conditions that may identify unforeseen
232 consequences resulting from unanticipated environmental stresses on bee populations (e.g.
233 Gill, Ramos-Rodriguez & Raine 2012). Such studies in our opinion are therefore a crucial
234 component of the regulatory framework.

235

236 **Acknowledgement**

237 This work was carried out in preparation for a large-scale field experiment on the impacts of
238 neonicotinoids on pollinators jointly funded by Bayer Crop Science and Syngenta. The
239 sponsors were not involved in the analyses or the writing of the paper, nor have they seen or
240 commented on this paper prior to its submission. M.R. was funded by the Swedish Civil
241 Contingencies Agency, Carl Tryggers Foundation for Scientific Research, the Royal
242 Physiographic Society, and the Swedish Research Council (330-2014-6439).

243

244 **References**

245 Becher M.A., Grimm V., Thorbek P., Horn J., Kennedy P.J. & Osborne J.L. (2014) BEEHAVE: a systems
246 model of honeybee colony dynamics and foraging to explore multifactorial causes of colony
247 failure. *Journal of Applied Ecology,* **51,** 470–482.

248 Bruce T.J.A. (2010) Tackling the threat to food security caused by crop pests in the new millennium.
249 *Food Security,* **2,** 133-141.

250 Bryden J., Gill R.J., Mitton R.A.A., Raine N.E. & Jansen V.A.A. (2013) Chronic sublethal stress causes
251 bee colony failure. *Ecology Letters,* **16,** 1463-1469.

252 CEB (2011) *Methode n°230: Method for the evaluation of side-effects of plant protection products on*
253 *honey bees (Apis mellifera L.).* edn. Association Française de Protection des Plantes.

254 Colin M.E., Bonmatin J.M., Moineau I., Gaimon C., Brun S. & Vermandere J.P. (2004) A method to
255 quantify and analyze the foraging activity of honey bees: relevance to the sublethal effects
256 induced by systemic insecticides. *Archives of Environmental Contamination and Toxicology,*
257 **47,** 387-395.

258 Cresswell J.E. (2011) A meta-analysis of experiments testing the effects of a neonicotinoid insecticide
259 (imidacloprid) on honey bees. *Ecotoxicology,* **20,** 149-157.

260 Cutler G.C., Scott-Dupree C.D., Sultan M., McFarlane A.D. & Brewer L. (2014) A large-scale field study
261 examining effects of exposure to clothianidin seed-treated canola on honey bee colony health,
262 development, and overwintering success. *Peerj,* **2,** 2167-8359.

263 de Krom M., Dessein J. & Erbout N. (2014) Understanding Relations between Science, Politics, and the
264 Public: The Case of a GM Field Trial Controversy in Belgium. *Sociologia Ruralis,* **54,** 21-39.

265 Defra (2014) *The National Pollinator Strategy: for bees and other pollinators in England*, edn. Defra,
266 Bristol, UK.

267 EFSA (2013) Guidance on the risk assessment of plant protection products on bees (Apis mellifera,
268 Bombus spp. and solitary bees). *EFSA Journal* **11:3295,** 266.

269 EFSA (In press) Draft scientific opinion: Scientific Opinion on the temporal and spatial ecological
270 recovery of non-target organisms for environmental risk assessments. *EFSA Journal***,**
271 http://www.efsa.europa.eu/sites/default/files/assets/150622c.pdf.

272 EPPO (2010) PP 1/170 (4): Side-effects on honey bees. *EPPO Bulletin,* **40,** 313- 319.

273 Gallai N., Salles J., Settele J. & Vaissière B.E. (2009) Economic valuation of the vulnerability of world
274 agriculture confronted to pollinator decline. *Ecological Economics,* **68,** 810-821.

275 Gill R.J., Ramos-Rodriguez O. & Raine N.E. (2012) Combined pesticide exposure severely affects
276 individual- and colony-level traits in bees. *Nature,* **491,** 105-U119.

277 Godfray H.C.J., Blacquiere T., Field L.M., Hails R.S., Petrokofsky G., Potts S.G., Raine N.E., Vanbergen
278 A.J. & McLean A.R. (2014) A restatement of the natural science evidence base concerning

neonicotinoid insecticides and insect pollinators. *Proceedings of the Royal Society B-Biological Sciences,* **281**.

Godfray H.C.J., Blacquière T., Field L.M., Hails R.S., Potts S.G., Raine N.E., Vanbergen A.J. & McLean A.R. (2015) A restatement of recent advances in the natural science evidence base concerning neonicotinoid insecticides and insect pollinators. *Proceedings of the Royal Society of London B: Biological Sciences,* **282**.

Godfray H.C.J., Crute I.R., Haddad L., Lawrence D., Muir J.F., Nisbett N., Pretty J., Robinson S., Toulmin C. & Whiteley R. (2010) The future of the global food system. *Philosophical Transactions of the Royal Society B-Biological Sciences,* **365,** 2769-2777.

Hallmann C.A., Foppen R.P.B., van Turnhout C.A.M., de Kroon H. & Jongejans E. (2014) Declines in insectivorous birds are associated with high neonicotinoid concentrations. *Nature,* **511,** 341–343.

HSE (2015) The Applicant Guide: Extrapolated And Assessed Experimental Approvals. *http://www.pesticides.gov.uk/guidance/industries/pesticides/topics/pesticide-approvals/pesticides-registration/applicant-guide/the-applicant-guide-extrapolated-and-assessed-experimental-approvals.htm#section_9point1point2. Accessed on 12/5/2015*.

Johnson R.M., Dahlgren L., Siegfried B.D. & Ellis M.D. (2013) Acaricide, fungicide and drug interactions in honey bees (Apis mellifera). *PLoS One,* **8,** e54092.

Kennedy C.M., Lonsdorf E., Neel M.C., Williams N.M., Ricketts T.H., Winfree R., Bommarco R., Brittain C., Burley A.L., Cariveau D., Carvalheiro L.G., Chacoff N.P., Cunningham S.A., Danforth B.N., Dudenhöffer J.-H., Elle E., Gaines H.R., Garibaldi L.A., Gratton C., Holzschuh A., Isaacs R., Javorek S.K., Jha S., Klein A.M., Krewenka K., Mandelik Y., Mayfield M.M., Morandin L., Neame L.A., Otieno M., Park M., Potts S.G., Rundlöf M., Saez A., Steffan-Dewenter I., Taki H., Viana B.F., Westphal C., Wilson J.K., Greenleaf S.S. & Kremen C. (2013) A global quantitative synthesis of local and landscape effects on wild bee pollinators in agroecosystems. *Ecology Letters,* **16,** 584-599.

Khoury D.S., Myerscough M.R. & Barron A.B. (2011) A Quantitative Model of Honey Bee Colony Population Dynamics. *PLoS ONE,* **6,** e18491.

Lundin O., Rundlöf M., Smith H.G., Fries I. & Bommarco R. (2015) Neonicotinoid insecticides and their impacts on bees: a systematic review of research approaches and identification of knowledge gaps. . *PLoS One* **10,** e0136928.

McDougall P. (2010) The Cost of New Agrochemical Product Discovery, Development and Registration in 1995, 2000 and 2005-8. R&D expenditure in 2007 and expectations for 2012 Final report. . *Comisioned by CropLife America and the European Crop Protection Association (ECPA)*.

Medrzycki P., Giffard H., Aupinel P., Belzunces L.P., Chauzat M.-P., Claßen C., Colin M.E., Dupont T., Girolami V., Johnson R., Conte Y.L., Lückmann J., Marzaro M., Pistorius J., Porrini C., Schur A., Sgolastra F., Delso N.S., van der Steen J.J.M., Wallner K., Alaux C., Biron D.G., Blot N., Bogo G., Brunet J.-L., Delbac F., Diogon M., Alaoui H.E., Provost B., Tosi S. & Vidau C. (2013) Standard methods for toxicology research in Apis mellifera. *Journal of Apiculture Research,* **54,** 1-60.

Mommaerts V., Reynders S., Boulet J., Besard L., Sterk G. & Smagghe G. (2010) Risk assessment for side-effects of neonicotinoids against bumblebees with and without impairing foraging behavior. *Ecotoxicology,* **19,** 207-215.

OECD (1998a) *OECD guideline for testing of chemicals. Test No 213: Honey bees, acute oral toxicity test.*, edn. OECD

OECD (1998b) *OECD guideline for testing of chemicals. Test No 214: Honey bees, acute contact toxicity test*, edn. OECD

Oerke E.-C. (2006) Crop losses to pests. *The Journal of Agricultural Science,* **144,** 31-43.

Pilling E., Campbell P., Coulson M., Ruddle N. & Tornier I. (2013) A Four-Year Field Program Investigating Long-Term Effects of Repeated Exposure of Honey Bee Colonies to Flowering Crops Treated with Thiamethoxam. *PLoS ONE,* **8,** e77193.

Potts S.G., Biesmeijer J.C., Kremen C., Neumann P., Schweiger O. & Kunin W.E. (2010) Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution,* **25,** 345-353.

Qi A., Perry J.N., Pidgeon J.D., Haylock L.A. & Brooks D.R. (2008) Cost-efficacy in measuring farmland biodiversity – lessons from the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. *Annals of Applied Biology,* **152,** 93-101.

Rundlöf M., Andersson G.K.S., Bommarco R., Fries I., Hederstrom V., Herbertsson L., Jonsson O., Klatt B.K., Pedersen T.R., Yourstone J. & Smith H.G. (2015) Seed coating with a neonicotinoid insecticide negatively affects wild bees. *Nature,* **521,** 77-80.

Tilman D., Fargione J., Wolff B., D'Antonio C., Dobson A., Howarth R., Schindler D., Schlesinger W.H., Simberloff D. & Swackhamer D. (2001) Forecasting Agriculturally Driven Global Environmental Change. *Science,* **292,** 281-284.

Vanbergen A.J., Baude M., Biesmeijer J.C., Britton N.F., Brown M.J.F., Brown M., Bryden J., Budge G.E., Bull J.C., Carvel C., Challinor A.J., Connolly C.N., Evans D.J., Feil E.J., Garratt M.P., Greco M.K., Heard M.S., Jansen V.A.A., Keeling M.J., Kunis W.E., Marris G.C., Memmott J., Murray J.T., Nicolson S.W., Osborne J.L., Paxton R.J., Pirk C.W.W., Polce C., Potts S.G., Priest N.K., Raine N.E., Roberts S., Ryabov E.V., Shafir S., Shirley M.D.F., Simpson S.J., Stevenson P.C., Stone G.N., Termansen M., Wright G.A. & Insect Pollinators I. (2013) Threats to an ecosystem service: pressures on pollinators. *Frontiers in Ecology and the Environment,* **11,** 251-259.

Wang M. & Brewer L. (2013) New computer methods for honeybee colony assessments. Pesticide Risks for Pollinators. 8th SETAC Europe Special Science Symposium, Brussels (ed.^(eds, pp.

Whitehorn P.R., O'Connor S., Wäckers F.L. & Goulson D. (2012) Neonicotinoid pesticide reduces Bumble Bee colony growth and queen production. *Science,* **336,** 351-352.

Williamson S.M. & Wright G.A. (2013) Exposure to multiple cholinergic pesticides impairs olfactory learning and memory in honeybees. *Journal of Experimental Biology,* **216,** 1799-1807.

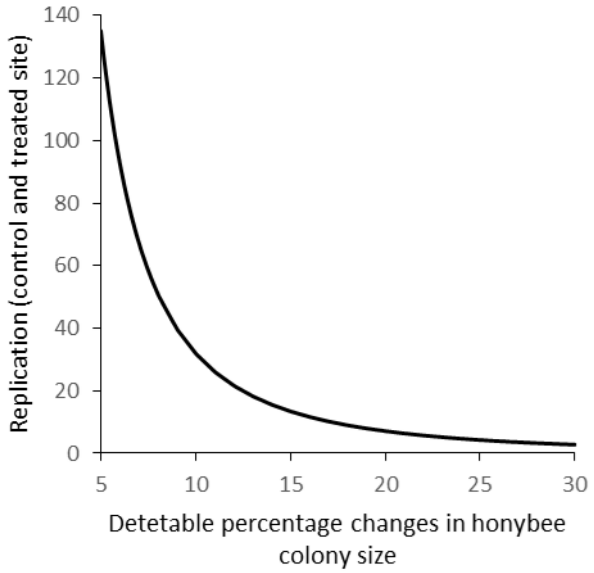
Supporting Information Appendix S1. Simple power analysis to determine replication for field-based studies identifying the effect of pesticides on honeybees.

Supporting Information Appendix S2: Derivation of the predicted costs of field scale evaluations of honeybees for regulatory studies.

Figure captions

Fig. 1. Relationship between experimental replication (control and pesticide treated field sites) and the detectable changes in honeybee colony (total number of bees) effect size based on power equations presented by EFSA (2013). For each effect size this the replication required to detect a response with a fixed 80% probability and a significance level of α=0.05. See Supplementary Material S1 for full details.

366 Fig. 1



367

368

369

370

371