‘Inert’ Ingredients Are Understudied, Potentially Dangerous to Bees and Deserve More Research Attention

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**Abstract**

Agrochemical formulations contain two broad classes of chemicals: active ingredients (AIs), which confer pest control action, and ‘inert’ ingredients, which facilitate the action of the AI. Most research into the effects of agrochemicals focusses on the effects of AIs. This reflects the assumption, engrained in legislation, that ‘inert’ ingredients are non-toxic. A systematic review of relevant research shows that for bees- a significant focus of research into agrochemical impacts, due to their essential role as pollinators- this assumption is both without empirical foundation and likely to be untrue. After conducting a systematic literature search, we found just 16 publications that actively tested the effects of ‘inert’ ingredients on bee health. In these studies, ‘inert’ ingredients were found to cause mortality in bees through multiple exposure routes. ‘Inert’ ingredients also acted synergistically with other stressors, and caused sublethal and colony level effects. In addition, the lack of research on ‘inert’ ingredient effects on bees is compounded by a lack of diversity in study organism used, with only 2 publications assessing effects on non-*Apis* bees. We argue that ‘inert’ ingredients have distinct, and poorly understood, ecological persistency profiles and toxicities making research into their individual effects necessary. We highlight the near total lack of mitigation in place to protect bees from ‘inert’ ingredients, and argue that research efforts should be redistributed to address the knowledge gap identified here. Specifically, we call for a new focus on testing the wealth of understudied ‘inert’ substances, and a shift away from testing well understood chemicals like neonicotinoids. If so-called ‘inert’ ingredients are, in fact, detrimental to bee health, their potential role in widespread bee declines needs urgent assessment.

**Introduction**

Ecosystem services provided by pollinators contribute $235-577 billion to the global economy each year, with bees providing the majority of pollination (Potts et al., 2016). Consequently, declines in bees pose a significant threat to this economic value (Potts et al., 2016), with, for example, 37% of EU bee species where trends can be measured showing declines (Nieto et al., 2014). While numerous factors are likely contributing to these declines, one factor that has been repeatedly linked to bee declines is the widespread use of pesticides, with experimental, correlational and modelling work at a range of scales confirming the link (Rundlöf et al., 2015, Woodcock et al., 2016, McArt et al., 2017). However, pesticides are not applied alone, but rather are used within complex formulations. Each formulation includes both the active ingredient (AI) itself, and co-formulants that facilitate the action of the AI (Hazen, 2000). When applied to crops, such formulations are often further accompanied by separate products called adjuvants that complement the action of the pesticide. These substances play a range of roles including surfactants which help AI penetrate into leaves, emulsifiers which help products stay thoroughly mixed, and solvents which help dissolve the active ingredient. Both co-formulants and adjuvants are referred to as ‘inert’ ingredients, because they are not intended to have direct pest control action.

There are no comprehensive figures for global ‘inert’ use, as California is the only regulatory zone to accurately record their usage (Mullin et al., 2016), but they are known to be heavily used across the globe. Under the American federal Environmental Protection Agency there are around 4000 ‘inert’ ingredients in use in America (Weinhold 2010). No equivalent data are available for the EU, but there are 294 separate adjuvant products and 2892 separate pesticide products registered for use in the UK alone (Health and Safety Executive UK, 2020a). As all AIs are applied as part of formulations, all formulations contain co-formulants, and formulations are commonly sprayed in a tank mix containing an adjuvant product, we can surmise that the quantity of ‘inert’ ingredient application is commensurate to, or likely even exceeds, that of AIs. Further, no mitigation measures are attached to ‘inert’ ingredients, meaning they can often be sprayed onto crops while bees forage on them, meaning bees exposure to them is considerable.

While regulatory bodies require AIs to undergo a suite of toxicity testing on bees (e.g., EPA, 1996, EC, 2009, EFSA, 2012), no parallel testing is required for individual ‘inert’ ingredients (EPA, 1996, EC, 2009), despite evidence of potential toxicity (Cox and Surgan, 2006, Mesnage, and Antoniou 2018). Instead, in the EU there is toxicity testing of a single commercial product per AI, called the ‘representative formulation’ (EC, 2013), while in the US only the active ingredient toxicity is considered (EPA, 1996, Mullin et al. 2015). In the EU at the national level, all other formulations with the same AI, of which there can be hundreds (Health and Safety Executive UK, 2020b) need individual approval. Which additional formulations trigger testing is determined by the similarity of their composition relative to already tested substances (Chemical Regulation Division, 2021). If the toxicity can be predicted based on existing data from formulations with a similar composition, then no additional testing is required. Formulations for which toxicity cannot be reliably predicted are not submitted to the full suite of ecotoxicological testing, but instead benchmarked against existing products using mortality at single dose to demonstrate equivalent toxicity (Chemical Regulation Division, 2021).

We argue that this regime is insufficient to protect bees. Firstly, the adjuvants that are added to tank mixes with these formulations undergo no bee toxicity testing at all (EPA, 1996, EC, 2009), nor any scrutiny of their ecotoxicology, meaning that there is no regulatory data affirming their safety to bees. An otherwise safe tank mix could become toxic to bees if the adjuvant added is toxic to bees (Moffet, Morton and MacDonald, 1972). Secondly, extensive data, including that collected by regulators, has demonstrated high variation in the toxicity of formulations with the same AI to bees (Straw, Carpentier and Brown, 2021, EFSA, 2015, Mullin 2015). Finally, regulatory testing regimes are tailored to detect toxicity from potent insecticides capable of causing short term mortality at low doses, not from ‘inert’ ingredients which are more likely to have more subtle, but still pertinent, sublethal effects at higher doses.

Our current understanding of the effects of ‘inert’ ingredients is almost exclusively centred around how they impact the toxicity of AIs (Mullin et al., 2015). This review will focus on the individual impacts of ‘inert’ ingredients, not how they impact AI toxicity, which while relevant is outside the scope of this review. However, it is important that we understand the effects of ‘inert’ ingredients in isolation because the ecological fate of each ingredient is unlikely to be uniform across the formulation (﻿Katagi, 2008).

Importantly, the development process of AIs makes them less likely to be ecologically persistent than ‘inert’ ingredients. In the development of AIs, specific attention is paid to their environmental persistence. Regulations like Maximum Residue Limits (MRLs) aimed at capping consumer exposure incentivise agrochemical companies to produce AIs that readily degrade. There are no MRLs for ‘inert’ ingredients (EC, 2009), and as such no pressure to produce fast decaying substances. One example of this is the AI class, the pyrethroids; cypermethrin, permethrin, and deltamethrin all have half-lives in pond water of <1 day (Tooby et al., 1981, Crossland, 1982, Rawn et al., 1982). In contrast, the surfactant adjuvant Multi-Film X-77, which may be applied as part of the same tank mix as pyrethroids, can repel honeybee visitation from a pond for 6 months after an initial spiking of 500ppm (Moffett and Morton 1973 and 1975). This concentration of Multi-Film X-77 also causes honeybees to drown at high rates for 60 days after application (Moffett and Morton 1973). In this scenario the pyrethroid AI has degraded well below the limit of detection whilst the ‘inert’ adjuvant is still causing significant mortality for months after. While not all AIs degrade as fast as pyrethroids, and not all ‘inert’ ingredients are likely to be as persistent as surfactants, this illustrates that assuming that all ingredients in a formulation will behave in a uniform manner once in the environment is unlikely to be true.

One of the reasons there is a paucity of data on the environmental fate or toxicity of ‘inert’ ingredients’ is that, under EU law only co-formulants with specific human hazard statements attached need to be reported as ingredients (EC, 2006). Despite this EU laws are among the most stringent in the world, with comparable documents from the US having even less information. The identity and concentration of other ingredients are explicitly protected under EU law as proprietary information (EC, 2009). Maintaining the identity of ‘inert’ ingredients as trade secrets severely impedes researchers’ capacity to understand how they spread in, and affect, nature (Chen, Fine and Mullin, 2018, Straw, Carpentier and Brown, 2021).

Given the widespread usage, uncharacterised ecological persistency and uncharacterised hazard of ‘inert’ ingredients, their application in the absence of specific regulatory testing or academic research makes them potential unidentified drivers of bee declines. An example illustrating why this could be the case is the neonicotinoids (imidacloprid, thiamethoxam and clothianidin), for which authorization for outdoor was revoked in the EU in 2013 (EC, 2013). These substances had undergone, and passed, full ecotoxicological testing (including assessment of risks to bees) prior to approval, but were nonetheless later shown through academic research to cause serious detriment to bees and bee populations, mediated through sublethal effects that the regulatory process failed to detect (Rundlöf et al. 2015 REFS). Just as the limited scope of the regulatory system failed to detect the risk neonicotinoids posed to bees, ‘inert’ ingredients too could be severely damaging to bees without triggering concern during the regulatory process. Consequently, academic research has a significant role to play in assessing the exposure, hazards and risks associated with ‘inert’ ingredients within pesticide formulations.

Existing academic research on ‘inert’ ingredients has focussed on two categories: surfactants (most commonly as adjuvants) and solvents (most commonly as co-formulants). Surfactants (short for **surf**ace **act**ive **a**ge**nt**), are the among the most common adjuvant type (Health and Safety Executive UK, 2020a). They function by reducing surface tension, enabling the spray to spread out over the surface of the leaf, increasing contact area (Stevens, 1993). A higher contact area improves penetration of the AI, improving efficacy of the spray (Stevens, 1993). Solvents are co-formulants that allow an AI to be dissolved at a higher concentration than if it were dissolved in water (Hazen, 2000). Because formulations are sold as concentrated stocks which are then diluted in water, this makes formulations cheaper to produce, distribute and store. Given that many AIs are poorly soluble in water, solvents are likely very common co-formulants. Crop oil concentrates are a much less frequently studied type of ‘inert’. They are typically petroleum-based spray adjuvants used to reduce droplet evaporation, and aid degradation of the wax surface on a leaf, which aids AI penetration. Here we use a systematic review approach to summarise what is known about the effects of ‘inert’ ingredients on bees.

**Methods**

Web of Science Core Collection and Google Scholar searches were undertaken based on the methods used by Cullen et al., (2019), using the PRISMA framework (Moher et al., 2009), and combined with forward and backwards citation tracing to ensure that all relevant literature was captured, although we do acknowledge that using only the English language potentially excludes relevant literature. The systematic review included only publications where there was an ‘inert’ only treatment group being tested on bees, or ‘inert’ ingredient residue data in honey, nectar, wax or pollen.

Peer reviewed publications were included in the review if they presented new experimental research testing at least one treatment of an agricultural co-formulant or adjuvant, with an appropriate control, or measured residues of an agricultural co-formulant or adjuvant in a bee or bee related medium, with the agricultural co-formulant or adjuvant being defined as such by the publications authors.

Literature was initially characterised by title, with titles lacking relevance to agriculture, bees or pesticides being excluded, then by abstract, with abstracts lacking relevance to the search criteria removed. Finally, remaining literature was read in full and the full exclusion criteria were applied. Throughout the search ambiguously titled publications were retained to the next stage. Papers not accessible online, despite all reasonable efforts made to acquire them were excluded.

Because the definition of adjuvant varies between authors it is defined here as meaning a tank additives without purpose of specific pesticidal action (regardless of organic/regulatory status). For instance, neem oil adjuvants which are marketed as insecticidal would be excluded. Because synergist co-formulants are included in insecticide formulations for their specific toxicity/immune inhibitory function to insects, despite being non-lethal themselves, they were excluded from the study as they are not intended to be biologically inert. Because solvents are often used in studies testing AI’s, they were only included in the review if the publication explicitly mentioned the solvent as being used in agricultural products, this is because the solvents used commonly by researchers are rarely the same substances used by the agrochemical industry.

The Web of Science Core Collection search was conducted in November 2020 using the following termsTopic, Title and Abstract Search = (((adjuvant\* OR coformulant\* OR co-formulant\* OR \*formulant\*) OR (Penetration OR "Odour mask\*" OR Stabiliz\* OR Preservative\* OR Surfactant\* OR Emulsifier\* OR Diluent\* OR Propellant\* OR Antifoaming OR Solvent\* OR Carrier\*)) AND (\*bee OR \*bees)). The Abstract search did not use wildcards before words because this functionality was not supported. A supplementary Google Scholar search was made to ensure all literature was captured with the terms ("bee" OR "bees") AND ("adjuvant" OR "coformulant" OR "co-formulant" OR "formulant"), with the first 200 publications searched in May 2020. Forward citation tracing was performed with Google Scholar in May 2020, as well as reverse citation tracing using the reference list. More comprehensive exclusion criteria, methodology and results are available in the Supplementary Material XX.

**Results and Discussion**

A total of 16 publications (from 1973 to 2019) fulfilled the inclusion criteria, comprising 12 experimental, 2 residue analyses and 2 mixed studies (Figure 1). There was a mixture of methodological approaches, with 8 laboratory studies, 4 semi-field and 4 field studies. However, diversity among study organisms was severely limited, with 14 studies testing honeybees, and just two studies on a species other than *Apis mellifera* (specifically, the solitary bees *Osmia lignaria* and *Megachile rotundata*). This demonstrates the lack of knowledge about how these widely applied substances could impact wild bee species.

Most studies (*n* = 11) tested surfactants, while some tested solvents (*n* = 5) and just one tested crop oil concentrates (*n* = 1). The life history stage studied varied, with adults being the most commonly studied stage (*n* = 11), followed by larvae (*n* = 5), and then pupae (*n* = 1) and eggs (*n* = 1). Nearly all studies focused on mortality (*n* = 12), while reproduction was the second most commonly studied metric (*n* = 3), followed by foraging ability, nesting ability and food consumption (*n* = 2 for all). Among the residue studies, two focussed upon solvents, and two surfactants. In total 54 substances or products have been tested in the academic literature, and just six have been tested in more than one publication indicating a lack of a depth of study for those tested. For further analysis of the publications included in this study, the metrics extracted from them, and additional quantitative results, see the Supplementary Material. While the frequency of publications has increased in recent years this is more likely to represent an increase in publications in general (Bornmann and Mutz, 2015), rather than increased interest in ‘inert ingredients’. It is also worth noting that seven of the publications dating from post-2010 are from one network of authors.



Figure 1. The number of studies testing co-formulants/adjuvants on bees which fulfil the inclusion criteria plotted against their year of publication.

The risk an agrochemical poses to bees is a combination of the exposure bees face and the hazard it poses to bees. Below the research found by the systematic review is divided into residue studies, which quantify exposure, and experimental studies, which quantify hazard.

**Residue studies**

Because the ecological persistency of ‘inert’ ingredients in nature is poorly understood we do not know how much bees are exposed to them. To address this question, it is possible to measure ‘inert’ ingredient residues in bee matrices such as honey, pollen, nectar and wax, however there are very few publications on this topic. What evidence is available has typically identified wax as a major substrate for residue accumulation. Two publications have looked at various surfactants, and two at the solvent N-Methyl-2-Pyrrolidone (NMP). Chen and Mullin (2013) developed a methodology for detecting trisiloxane surfactants in honeybee matrices, using the QuEChERS (quick, easy, cheap, effective, rugged, and safe) approach. Trisiloxane surfactants are common surfactant co-formulants in the organosilicone group, and are included in notable spray adjuvants like Silwet L-77 and Dyne-Amic. They can be used in a range of pesticide classes, and on a range of crops. Honey, pollen and wax samples were taken from seven US states. There were no positive detections in honey, but 60% of pollen and all wax samples had positive detections (max. concentrations 39ppb and 390ppb respectively). These authors later tested for nonylphenol ethoxylate and octylphenol ethoxylate surfactants in the same matrices (Chen and Mullin, 2014), finding nonylphenol ethoxylate surfactants in all samples. Again, honey was the least contaminated (46±26ppb, mean ± standard deviation), followed by pollen (429±203ppb) and wax (1051±2897ppb). Octylphenol ethoxylate surfactants were less prevalent and when present at a lower average concentration. These studies demonstrate that bees are exposed to surfactants at non-trivial concentrations, however whether these concentrations have a meaningful impact on bee toxicity is unknown, particularly as the experimental literature covered below typically uses much higher concentrations.

NMP, which is a solvent co-formulant often used in insecticide formulations, is the only ‘inert’ ingredient to have been purposefully applied to a crop to enable the explicit measurement of residues in pollen or other bee relevant matrices (Fine et al., 2017). Applying the product directly then measuring residues allows for the measurement of the initial peak concentration prior to degradation to background levels, useful in informing worst case scenario exposure regimes in laboratory experiments. An insecticide formulation (Rimon 0.83EC) containing 40-50% NMP was applied following manufacturer’s instructions to apple trees either at the bud stage or while flowering. The likelihood of detection of NMP residues varied with time after application, and flower stage. In crops sprayed at bud, a high of 22,000ppb (17,150 ± 4,390ppb) in pollen was detected 12 hours after application. A smaller, lab-based pilot study with application direct to the flowers found a high of 234,600ppb in pollen 2.5 hours after application (Fine et al., 2017). These residue levels are very high in comparison to those of insecticidal compounds. For example, novaluron, the insecticide AI in the formulation applied was found at a high of 4,070ppb when sprayed at the flowering stage. This is 58x less concentrated than the highest NMP measurement. While these results are not directly comparable because the methods differed slightly between experiments, they nonetheless illustrates that bees exposure to NMP is comparably very high.

The experimental work testing NMP, which is discussed in the next section, has exposed bee through nectar, while the residue work has measured NMP in pollen, as such it is difficult to assess the field realism of the exposure regimes in the experimental work. NMP residues within experimentally exposed workers and larvae have also been assessed (Fine and Mullin 2017), finding larvae are less capable metabolising the substance.

For AIs, exposure regimes are typically designed with reference to the results of semi-field studies where the pesticide is deliberately applied to a crop, usually the *Phacelia tanacetifolia*. Pollen and nectar brought back to the nest by foraging honeybees is collected and measured over time. Using these data, chronic exposure scenarios can be constructed which assess the potential effects on individuals or colonies of bees foraging on a recently sprayed crop. Without these experiments for a range of ‘inert’ ingredients, it is not to inform exposure regimes with real world data.

If regulatory bodies mandated residue analysis for all agrochemicals, including ‘inert’ ingredients, we would have a better understanding of the complex exposure bees face to a diverse range of chemicals. Without residue analysis it is not possible to know whether current mitigation measures keep ‘inert’ ingredients within safe limits for non-target organisms. The kind of well-funded and systematic approach to residue monitoring required is something only a regulatorily mandated process can offer. Without this academic researchers will not be able to properly assess whether their exposure regimes are field-realistic, which could lead to over, or under-estimates of the risks that ‘inert’ ingredients pose to bees.

**Experimental studies**

There is only one full scale field study looking at ‘inert’ ingredient residues after application of a product (Fine et al., 2017) and background residue data are scarce. So typically the only reference point we can use for exposure regimes is the in-tank mix concentration, which is the concentration of the ‘inert’ ingredient in the solution sprayed. For co-formulants this is not always known because their concentration and identity are not required to be publicly disclosed (EC, 2009). For adjuvants most labels mandate a maximum concentration of 1% (10,000ppm). This means that without bioaccumulation we would expect that around 10,000ppm to be the very upper end of field realistic exposure, equivalent to feeding directly on in-tank mix. This is appropriate for acute exposure (see Ciarlo et al., 2012), but is likely to vastly overestimate field realistic chronic exposure. As such, the studies detailed below uses a range of values which may or may not be field realistic, regardless of this they elucidate the relative hazard the substances pose. While little is known about ‘inert’ ingredients ecological persistencies and how this maps to the ecotoxicological risk posed to non-target organisms like bees, we have known for nearly a century that surfactants have strong insecticidal action.

Soaps, which are surfactants, have been recognised as posing risks to insects as far back as 1931. "Insecticidal soaps are the oldest of recognized insect destroyers. Almost any form of soap, if used in a strong enough mixture, will kill soft-bodied insects” (Sanderson and Pearis, 1931, cited in Wolfenbarger 1957). The mechanism through which surfactants cause mortality in insects is unresolved, although Stevens (1993) notes that insect spiracles are similar in size to plant stomata, which surfactants are designed to penetrate. Thus, surfactants may inadvertently block the breathing apparatus of the insects and cause them to drown.

Adjuvants have been tested since the 1970s (Moffett and Morton 1973 and 1975), these studies found significant effects of surfactant adjuvants on honeybee drowning events when added to the bees’ water supplies, and commensurate repellence from the spiked water. When 1000ppm of Multi-Film X-77 was added to the water supply bucket for caged honeybee colonies 2,331 honeybees drowned in a single week which almost killed the colony, compared to 51 in the control. The same result was observed with free flying bees and pond water spiked with 500ppm. These visitation rate studies using ‘inert’ ingredients have never been repeated, meaning we do not know if the new generations of ‘inert’ ingredients could similarly be causing honeybee drownings. Moffett and Morton (1975) then expanded upon the repellence seen in the prior study, finding that the same substances could repel honeybee visitation to water sources for up to six months but did not deter visitation to sprayed flowers.

Exposure to adjuvants is not limited to contamination of water sources, as farmers spray adjuvants in a range of situations, and labels do not include any guidance for reducing bees’ exposure. As such, label guidance allows for direct overspray of bees which could cause mortality through contact exposure. Contact exposure occurs when a bee is exposed to spray droplets of a pesticide, or when it lands on a recently sprayed surface such as a flower or leaf. In experimental studies, this is often simulated by either using a spraying apparatus to mimic direct overspray of bees, or by pipetting 2µL of the pesticide onto the dorsal side of the thorax/abdomen of anesthetized bees (OECD, 214). Using a Potter spray tower, which replicates recommended spraying apparatus, two surfactant adjuvants, Pulse® and Boost®, were found to cause 100% mortality in honeybees at 40-50% of the label recommended concentration (Goodwin and McBrydie, 2000). The use of a Potter spray tower and use of label recommended concentrations makes this study representative of in-field application. This suggests that these substances are highly likely to be driving mortality in the field given that bee exposure to surfactant adjuvants is high.

When testing the toxicity of surfactants as adjuvants, the methodology chosen is likely to influence the size of the observed effect. The standard contact toxicity test for honeybees, OECD 214, has been used to determine the toxicity (hazard) of both Silwet L-77 and Triton X-100, with LD50’s of 357µg and 1436µg respectively (Chen et al., 2019). This can be used to informs risk management strategies by allowing comparison of the toxicity with other substances. Donovan and Elliott, (2001) used OECD 214 to test the toxicity of several surfactant adjuvants on honeybees and found no significant mortality from any substance. However, the dosing regime lacked the range needed to detect lethal effects and is insufficient to justify their conclusion that the substances tested were ‘non-toxic to honey bees’.

Chronic oral toxicity of surfactants has been tested on honeybees in two studies. Moffett and Morton (1973) found two out of seven adjuvants/surfactant co-formulants to cause mortality at the very high rate of 1000ppm (0.1%) in nectar. At 100 and 10ppm, no significant difference was detected from the control even over the full 60-day exposure period. In contrast, Chen, Fine and Mullin (2018) suggested that three trisiloxane surfactants at 100ppm reduced survival over an 8- or 10-day period. There was a clear effect of the class of surfactant, with trisiloxane surfactants causing >90% mortality relative to the control, while alkylphenol polyethoxylates and fatty amine polyethoxylates surfactants caused less than 20%. These results indicate that the hazard surfactants pose could be potentially mitigated by redesigning formulations/adjuvants to choose the safer options.

However, the effects of pesticide are not limited to mortality, and only when we consider wider metrics like individual and colony reproductive success can we truly assess the impact of a pesticide (Straub, Strobl and Neumann, 2020). For example, impairment of learning ability may impact upon foraging efficiency (Raine and Chittka, 2008), which may then impact colony reproductive success. Ciarlo et al. (2012) tested acute 20µg doses of several adjuvant products individually on honeybee learning using the proboscis extension reflex methodology. In the field, a honeybee feeding for just two seconds on sprayed tank mixture (which can be sprayed onto flowering crops or weeds) is enough to achieve a 20µg dose of the surfactant adjuvants tested. All 20µg doses of surfactant adjuvants impaired learning, but crop oil concentrates did not, suggesting that the different classes of ‘inert’ ingredient are toxicologically distinct.

Another important sublethal effect is queen rearing success, with reduced queen production being likely to reduce colony fitness in social bees. However, in the only study so far to examine this question, Johnson and Percel, (2013) found no effect of the surfactant adjuvant Break-Thru, at 200ppm in pollen, on several metrics of honey bee queen rearing success. The use of pollen as the exposure source in this study is important, as it represents a significant exposure route for developing bee larvae [ref]. Understanding how residues vary between nectar and pollen (Thompson et al., 2014; Zioga et al., 2020), and how this may impact different life-stages of bee, remains a largely open question.

While the studies described above have looked at ‘inert’ ingredients individually, pressures on bee health are multifactorial (Bryden et al., 2013), with novel stressors like agrochemicals adding to pre-existing stressors like parasites. Consequently, we may only be able to appreciate the impact pesticides have when we understand how they interact with other stressors. In only one publication has the interaction between an ‘inert’ ingredient and a stressor other than another agrochemical been tested. In a fully crossed experimental design Fine, Cox-Foster and Mullin (2016) spiked honeybee larval diets with 10ppm of the surfactant adjuvant Sylgard 309 and a representative dose of a mixed virus inoculum. The surfactant adjuvant was found to increase black queen cell viral titre significantly, demonstrating a interaction between the stressors. Both stressors alone reduced larval survival, causing failed moults, melanisation and other developmental abnormalities. When combined the stressors acted synergistically, causing more larval mortality than the additive impacts of either stressors relative to the control.

The systematic review conducted returned no publications on bumble bees (*Bombus spp.*), which is alarming given their agricultural and cultural importance [ref needed]. Only two publications have tested the effects of ‘inert’ ingredients on bee species other than honeybees, with both testing solitary bee species. Ladurner et al. (2008) tested the effects of the surfactant adjuvant Dyne-Amic on *Osmia lignaria* nesting behaviour and reproduction and reported no lethal or behavioural effects of Dyne-Amic. In contrast, Artz and Pitts-Singer (2015) tested the effects of the surfactant adjuvant N-90 on both *O. lignaria* and *Megachile rotunda* when sprayed on *P. tanacetifolia* and *Sinapis alba* at label-recommended rates. In flight cages with the sprayed crops, nest recognition ability in both species was significantly impaired by N-90. While no mortality was found, these results are likely to be conservative, as the N-90 spray was applied at night when bees were not foraging, whereas label guidance for N-90 do not mandate night application (N-90 Label), and so realistic field usage may result in direct contact with the spray, rather than residues which may have dried by the time bees become active. For similar substances direct contact can cause significant mortality, as seen in Goodwin and McBrydie (2000).

Beyond the research into surfactant adjuvants listed above, (and Ciarlo et al., 2012 which tested the effects of crop oil concentrates on honey bee learning ability) the only other groups of ‘inert’ ingredients tested have been the two solvents N-Methyl-2-Pyrrolidone (NMP) and Dimethyl Sulfoxide (DMSO). These solvents are alternatives to one another with one producer of DMSO advertising it as safer and less toxic than NMP (Gaylord Chemical Information Sheet). Promotional literature for DMSO also advertises that its low toxicity means it does not have to feature as a listed co-formulant in the EU. Both NMP and DMSO are widely used solvents in agricultural formulations (Zhu et al., 2014, Gaylord Chemical Information Sheet).

All work on oral exposure to NMP has used a chronic feeding regime whereby NMP was administered through sucrose. As no residue analyses of field realistic NMP nectar concentrations are currently available, a wide range of concentrations (100- 20,000ppm) have been used in the literature. The first study to assess NMP toxicity to honey bee larvae was Zhu et al. (2014), which found 50% mortality within 12 hours at 10,000ppm; however, in the absence of a control, these results cannot be interpreted (it is for this reason the publication is omitted from the systematic review results, which requires an appropriate control). When repeated, in a study by Fine et al. (2017), 100ppm of NMP caused significant larval mortality compared to the control, although mortality did not reach 50% over the 20-day trial period. In contrast, adult honeybees only experienced significant mortality at doses as high as 5000ppm treatment (Fine and Mullin 2017), which is unlikely to be a field realistic chronic exposure. This suggests that larvae are more susceptible to NMP than adults. The effects of chronic exposure to 500ppm NMP for 7-10 days on honeybee colony health was also investigated by Fine et al. (2017). This dose is above the 100ppm which is known to cause larval mortality, but below the 5000ppm which causes adult mortality. In this study, NMP inhibited colony weight gain and emerging forager counts, which is most likely to be caused by larval mortality and knock-on effects on colony foraging.

To investigate whether higher impacts of NMP on larvae were a function of differential detoxification, Fine and Mullin (2017) fed honeybee workers and larvae 200ppm NMP for six days and quantified residues of the NMP and its metabolites from the adults and larvae. They found that larvae were less able to detoxify the NMP, and this may explain the higher sensitivity of larvae to NMP.

Using OECD 214, NMP was found to have an acute contact LD50 greater than 2000µg per honeybee (OECD, 1998. Chen et al., 2019). This finding suggests NMP is of negligible toxicity when applied via acute contact.

DMSO has received less attention than NMP, with only two publications assessing its toxicity to bees. Moffett and Morton (1973) found that DMSO produced no significant lethal effects in honeybees with chronic exposure of 1000ppm for 60 days. Milchreit et al. (2016) found mixed effects of chronic oral exposure (500ppm) on honeybee brood development, with no detriment to fitness clearly demonstrated. Together, these results support the producer’s assertion that this substance is less toxic than its alternative NMP (Gaylord Chemical Information Sheet). If this is substantiated in directly comparable trials, DMSO could be used to replace NMP as a solvent to reduce the toxicity of pesticide formulations to bees.

**A call to reprioritise research into ‘inert’ ingredients**

Research into the effects of pesticides on bees is disproportionately focussed on AIs, with ‘inert’ ingredients receiving significantly less attention. The lack of attention given to ‘inert’ ingredients is most clearly visible when considering the number of publications focussing on them relative to the best studied pesticide class, insecticides. For example, a single AI, the neonicotinoid imidacloprid, was the subject of 168 publications as of 2015 (Lundin et al., 2015). This dwarfs the literature on ‘inert’ ingredients, with the systematic review here finding just 16 publications. The allocation of research is partially explained by insecticides intended purpose, with insecticide literally meaning a substance used to kill insects. However, despite ‘inert’ ingredients not being designed to kill insects, they can have unintended consequences on bee health as detailed above.

If bee ecotoxicological research is an applied science with the aim of understanding the risks pesticides could pose to bees, the optimal allocation of research effort to substances should match the potential risk each substance poses. This risk is a combination of the hazard posed to bees and the likelihood of exposure. The hazard is likely greatest with insecticides. However, exposure is likely to be greatest with ‘inert’ ingredients that are used in far higher quantities (Mullin et al., 2015), with little in the way of exposure mitigation. For example, whenever a formulation is applied, ‘inert’ ingredients are also applied as co-formulants. So, when a formulation has no label guidance aimed at reducing bee exposure to the AI, this means that exposure of bees to its co-formulants is also uncontrolled. The exposure bees face to ‘inert’ ingredients can be exemplified by the Environmental Information Sheet for one such formulation, Roundup® ProActive, which states “Roundup ProActive is of low toxicity to honeybees; there is no requirement to avoid application of the product when bees are foraging on flowering weeds.”. The current allocation of research effort has focussed very strongly on the hazard posed by insecticides, without recognising that ‘inert’ ingredients have vastly higher exposure levels (~~Supplementary Materials OR Box OR Table~~). This means that the allocation of research is primarily based on hazard, not risk as it should be.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Hazard** | **Exposure** | **Risk** |
| **Insecticide** | High | Low-  Stringent mitigation measures | Intermediate |
| **‘Inert’ ingredients** | Poorly characterised but non-negligible | Very high-  Little to no mitigation measures | Intermediate |

Table 1. Detailing the hazard, exposure and risk insecticides and ‘inert’ ingredients pose to bees. Risk = Hazard \* Exposure.

As illustrated in Table 1, while the hazards and exposures of insecticides and ‘inert’ ingredients differ, their risk to bees could be equivalent. As such, research effort should be reallocated to characterise their exposure and hazard to bees, after which the benefits of further research can be evaluated.

To be clear, what is necessary is not a cessation of research into insecticides, but instead a reallocation of resources to better reflect the risks bees face in the wild. Applied bee pesticide research would benefit from allocating resources to agrochemicals in proportion to their potential risk to bees. This would require a vast amount of research into large numbers of chemicals which may have never been tested on bees before. We propose that the potential, and likely impacts of these widely-applied substances on bee health represents a key knowledge gap that urgently requires research attention and funding.

~~To illustrate the difference in exposure between insecticides and ‘inert’ ingredients, let us consider the label guidance for a representative insecticide formulation (Closer®) and a representative mixture that contains “inert” ingredients (Roundup® ProActive and the surfactant adjuvant Newmans T-80™). For the exposure to the insecticide formulation we will focus on the AI (sulfoxaflor), and for the ‘inert’ mixture we will focus on the ‘inert’ ingredients which are co-formulants in the Roundup®, including surfactants (Mesnage et al., 2018) and the surfactants in the adjuvant T-80™. The restrictions which reduce bee exposure to either mixture are detailed below in Table 1, where it is clearly shown that little to no mitigation of exposure takes place for ‘inert’ ingredients.~~

|  |  |
| --- | --- |
| **~~Insecticide:~~**  **~~Closer®~~** | **~~‘Inert’ Ingredient Mix:~~**  **~~Roundup® ProActive (Contains Co-Formulants) +~~**  **~~Newmans T-80 (Adjuvant)~~** |
| ~~A maximum application rate of 24g/ha of the AI sulfoxaflor~~ | ~~A maximum application rate of 1200g/ha of the co-formulant surfactant Alkylpolyglycoside and 1600g/ha of the adjuvant ethoxylated tallow amine~~ |
| ~~A maximum of two applications per crop~~ | ~~No restrictions on repeated sprays~~ |
| ~~No application when bees are foraging and no application near flowering weeds~~ | ~~Can be applied directly onto bees or bee attractive flowering weeds~~ |
| ~~Can only be applied at a BBCH (plant development) stage well prior to flowering to reduce floral residue.~~ | ~~No BBCH (plant development) stage restriction~~ |
| ~~Must be applied outside of bee daily foraging activity~~ | ~~No restrictions on timing of application regarding bees~~ |
| ~~Must avoid application near environmental water~~ | ~~Can be applied directly into enclosed waters (excluding the T-80™)~~ |

~~Table 1. Comparison of mitigation measures for insecticides versus ‘inert’ ingredients.~~

**Conclusion**

The literature reviewed above raises a number of concerns around the impacts of ‘inert’ ingredients on bee health and productivity at the individual and colony levels. What little research we have on ‘inert’ ingredient residues in nature shows them to be widespread, and at high concentration (Chen and Mullin, 2013, 2014, Fine et al., 2017), although our understanding of what the normal concentration range of ‘inert’ ingredients is in agricultural systems is underdeveloped. More research into the environmental pervasiveness and persistence of ‘inert’ ingredients would inform future experimental research on appropriate dosing regimes and expand our understanding of the risk they pose to bees. Importantly, and in addition to this limited understanding of environmental residues, the research identified here demonstrates that ‘inert’ ingredients are not ecotoxicologically benign, and as such they should be subject to greater regulation.

‘Inert’ ingredients drive mortality through multiple exposure routes, synergise with other stressors and cause sublethal effects. While we call on regulators to require testing of ‘inert’ ingredients on bees, we also caution that the current regulatory testing system is ill-equipped to test the effects of ‘inert’ ingredients. Current regulatory testing exclusively uses methodologies designed for neurotoxic insecticides, which may not properly characterise the risks of ‘inert’ ingredients. The finding that surfactants increased pond drownings (Moffett and Morton, 1973, 1975) demonstrates the need for risk assessments to be more tailored to the risk posed by each unique substance. Current regulatory testing focuses heavily on mortality, at a cost to a more fitness-focused where the whole lifecycle and reproductive success of bees is considered (Straub, Strobl and Neumann, 2020). Sublethal effects caused by surfactants (Ciarlo et al., 2012, Artz and Pitts-Singer 2015), that could feed into fitness impacts, would be missed by the current regulatory system. Similarly, given that synergistic effects of surfactants have already been identified (Fine, Cox-Foster and Mullin, 2016), a testing approach that incorporates multiple stressors is essential.

‘Inert’ ingredients not only interact with other stressors, but with AI’s also. ‘Inert’ ingredients are designed to facilitate AIs toxicity to target organisms, however, there is significant research that they also increase the toxicity of AIs to non-target organisms as reviewed in Mullin et al. (2015) and Nagy et al. (2020). A systematic comparison of AI toxicity versus whole formulation toxicity covering academic and regulatory data would give highly informative results, but is outside of the scope of this systematic review. As prior reviews have demonstrated, formulations are commonly more toxic to non-target organisms suggesting that the term ‘inert’ ingredients may not be appropriate.

The use of the words ‘inert’ or ‘inactive’ to describe co-formulants and adjuvants posits that they are toxicologically benign substances. The research collated here demonstrates that this is not true for all such substances and highlights a lack of data for many more. As such we would suggest that the terms ‘co-formulant’ or ‘adjuvant’ where appropriate are better descriptors of the substances because they are neutral regarding their toxicological activity.

While the language used to describe ‘inert’ ingredients does not reflect their potential toxicity, the legislation regulating them also does not reflect this. Legislation that protects formulation composition as trade secrets hampers research into the impacts of ‘inert’ ingredients (EC, 2009, Mullin et al., 2015), as such publication of formulation composition would be a critical step forward for environmental risk assessment. Further, full disclosure of ingredients would improve transparency and build trust for both consumers and farmers (Mullin et al., 2015).

In conclusion, evidence of ‘inert' ingredients having the potential to cause mortality in bees dates back to the 1970’s (Moffett and Morton 1975), yet in the EU there is still no regulatorily mandated toxicity testing of ‘inert’ ingredients (EC, 2009). This means the only research stream is academic testing, which has produced just 16 publications to date. This represents a large gap in our understanding of pesticide ecotoxicology. The research collated here demonstrates that ‘inert’ ingredients are not inert and can pose significant and pertinent risks to bee health. We call on researchers to devote more attention to ‘inert’ ingredients and regulators to require testing of ‘inert’ ingredients to ensure their safety to bees.

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Cut out

These residue levels are very high in comparison to those of insecticidal compounds. For example, sulfoxaflor a substances touted as a successor to neonicotinoids, has been found to have peak residue levels of 5,190ppb when sprayed on flowers (EFSA, XXXX). This is 45x less concentrated than the comparable NMP measurement. While there are differences between the study method, this nonetheless illustrates that bees exposure to NMP is potentially very high.