ネオニコチノイド系農薬、ハチの異変、花粉媒介者サービスの持続性

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概要

20 年未満で、ネオニコチノイド系農薬は世界市場シェア 25%を超える最も広く使用されている殺虫剤の種類となった。花粉媒介者にとってこれは農薬の風景を塗り替えることとなった。これらの化学物質は神経伝達物質アセチルコリンを模倣し、昆虫にとって毒性の高い神経毒となる。ネオニコチノイド系農薬の浸透性作用は師部および木部にまで農薬が達し、結果的に花粉や花蜜にまで輸送する。土壌や水中において難分解性で、次の作物や野生植物にまでとりこまれる可能性のあるネオニコチノイド系農薬が広く使用され、花粉媒介者の体内に吸収され、ほとんどの年において亜致死濃度となる。ミツバチの巣に頻繁にネオニコチノイド系農薬が存在する結果となる。ネオニコチノイド系農薬は、フィールドでの現実的な使用量で、給餌行動がうまくいかなくなる、蜂児、幼虫の発達、記憶、学習、中枢神経システムへのダメージ、病気にかかりやすくなる、巣の衛生状態が悪くなるなど、様々な亜致死の悪影響をミツバチとマルハナバチのコロニーに引き起こす。ネオニコチノイドは様々な他の農薬により毒性が増幅し、ノゼマ原虫などの伝染性物質を相乗的に強め、蜂群崩壊を共に引き起こす。限られたデータから、他の野生の昆虫花粉媒介者に同様の毒性を示す可能性があることが示唆される。ネオニコチノイドの生産は現在も増えている。それゆえ、花粉媒介者の生態学的サービスを持続的なものにするためには、ネオニコチノイドに替わる花粉媒介者に優しい代替物に変換していく必要がある。

序論

1990 年代初めにイミダクロプリドとチアクロプリドが市場に出回り、ネオニコチノイド系農薬の害虫コントロール時代の幕開けとなった[1]。浸透性であり、この新しい神経毒を持つ殺虫剤は、植物、主に根から吸い上げられ、師部、木部の輸送を通じて植物の全ての部分に輸送する[2]。この浸透性の特徴および昆虫への高い毒性により、ネオニコチノイド組成は土壌処理や種子処理用の典型的な用量である 10 から 200g ha^{-1} で植物全体を長時間害虫から守るのに充分となった。

ネオニコチノイド系農薬は昆虫の中枢神経システムのニコチン性アセチルコリン受容体（nAChRs）と相互作用する。主に標的種のシナプス後細胞膜にあるニコチン性アセチルコリン受容体（nAChRs）のアゴニストとして作用し、高い親和性で結合することより、生来の神経伝達物質アセチルコリンを模倣する[3-8]。これが神経細胞の過度の興奮を引き起こし数分以内で昆虫を死に導くことができる[6,9]。ネオニコチノイド系農薬のいくつかの主要な代謝物も同様に神経毒であり、同じように受容体に作用するため[10-12]、浸透性殺虫剤として長く効果を発揮する。脊椎動物の神経システムのニコチン性アセチルコリン受容体（nAChRs）は結合部位が昆虫とは異なり、一般的にはネオニコチノイド系農薬に高い親和性を持つニコチン性受容体の数が少ないことが、脊椎動物よりも昆虫に選択毒性があると言われている理由である[9,13]。

現在市場に出回っている主なニコチノイド系農薬は、イミダクロプリド、チアメトキサム、クロチアニジン、チアクロプリド、ジノテフラン、アセタミ

プリド、ニテンピラム、スルホクサフルロルである[12,14,15]。導入以来、ネオニコチノイド系農薬は2010年農薬市場のマーケットシェア26%を占めるなど[16]、最も広く使用され、最も早く増加した種類の農薬である。2008 年に世界で2番目に多く使用されたのがイミダクロプリドである[17]。世界のネオニコチノイド系農薬の生産は現在も増加している[18]。2004 年頃から欧米で大規模使用が始まった。ネオニコチノイド系農薬は現在 120 カ国において[19]、ジャガイモ、米、トウモロコシ、砂糖大根、穀物、菜種油、ひまわり、果物、野菜、大豆、観葉植物、苗木、輸出用の種、綿花など1000 以上の用途で使用許可されている。

種子処理として使用される場合、使用された量の 1.6–20%のみしか作物に浸透しない。[20]残りの 80 – 98.4%は植物の害虫を守るために意図した作用をすることなく、環境を汚染する。 ネオニコチノイド系農薬の特性により、殺虫剤の環境中への拡散と変換は様々な環境濃縮や生物活性を起こす [21]。ネオニコチノイド系農薬は高い浸出性の特徴があるため、地表水と地下水を汚染する傾向にある[22–25]。

土壌と堆積物中の有機物への吸着により[24,26], 土と水分の平衡分配は土のタイプにより様々だが、典型的には 1 対 3 (log P = 0.57)である[25]. モニタリングデータのある国では、地表水から高いレベルのネオニコチノイド系農薬汚染が報告されている[27–30]。オランダでは1998 年と 2003 年から 2009 年の間の定期的水質モニタープログラムによる国内801の異なる場所でとられた 9037 つの水のサンプルのうち 45%が 13 ng l–1 のイミダクロプリド水質基準を超しており、平均濃度は 80 ng l^{-1}、最大濃度は 320 μg l^{-1} で、これはミツバチの急性毒性を示す値である[27]。アメリカでもネオニコチノイド系農薬は地表水で見つかっている。サザンハイプレーンのプラヤ湿地で 2005 年に集められた 108 の水のサンプルではチアメトキサムが平均 3.6 μg l–1 の濃度で、アセタミプリドが 2.2 μg l^{-1} の濃度だった [30]。

ネオニコチノイドとその代謝物は土壌、水中の堆積物、水の中で持続性がある。例をあげよう。イミダクロプリドのみを 1 回使用した6年後にシャクナゲの花(Rhododendron shrub blossoms)に 19 μg kg^{-1} の残留があった[31]。クロチアニジンの土壌での半減期は 148–6,900 日で[32], イミダクロプリドは 40–997 日 である[33]。結果的に、ネオニコチノイド系農薬は繰り返し使用されることで土に蓄積する可能性があり[23] 使用後少なくとも2年間は次の作物にも吸収される可能性がある [34]。サンプリングの1年もしくは2年前に種子処理されたコーンを使用した未処理の畑で採取された 33 の土壌サンプルの 97%からイミダクロプリドが見つかった[34]。土壌サンプルの濃度は 1.2 から 22 μg kg^{-1} の範囲だった[34]。いくつかの 研究ではネオニコチノイド系農薬を使用した畑の近くの野生の花からも農薬を検出している[35,36]。しかしながら、野生の花が、どの程度が汚染された土壌や水から浸透したネオニコチノイド系農薬を吸い上げているのか、あるいは種まき機のホコリにより花が汚染されているのかには知識ギャップがある。

ネオニコチノイド系農薬の導入当時は、有機リン系農薬やカーバメート系農薬にとって代わる効率的な農薬であると予測されていた[37]。 種子処理剤としては少量で済み環境をこれまでより汚染しないと言われていた。しかしながら、関係するのは量ではなく、非標的種への毒性、持続性、生体利用効率(bioavailability)につながる、害を引き起こす能力の大きさである。実は、ネオニコチノイド系農薬が使われて初めてすぐに花粉、花蜜、種まきに伴うホコリなどに残留するネ

オニコチノイド系農薬に非標的花粉媒介昆虫が暴露していることが明らかになった。これが様々な有害な効果をもたらす [10,37-43]。

花粉媒介者の生態系でのサービス

多様な花粉媒介者の中で [44] ハチは最も重要である。ハチの研究のほとんどは飼育下にあるセイヨウミツバチに集中しているが、25,000種類以上の異なるハチの種が確認されている。(FAO: Pollination; URL: http://www.fao.org/agriculture/crops/core-themes/theme/ biodiversity/pollination/en/). ハチは不可欠な生態系サービスを供給し、生物多様性の維持食品・繊維生産に重要な役割を担っている [45-51]。授粉は地球上の植物、野生動物、ヒトの快適な生活をつなぐ相互作用の総合システムを成している[52]。地球上の花が咲く植物全てのうち、87.5%が動物による授粉に利益を得ている [53]. 地球上では、 87 の主要な食用作物（世界の食品生産量の 35%にのぼる）が動物による授粉に頼っている[45]。花粉媒介者に頼っている作物はヒトの食品供給において不可欠な栄養素を担う重要なカギである[54]。養蜂業の歴史は農業が始まる前にさかのぼり[55,56] その後に農業と共に発展した [57,58]. 加えて、野生のハチは農業あるいは野生の花に相当な量の、そして多くの場合評価されていない授粉サービスを行っている[59,60]。 ハチやハチ製品は、薬理学的[61,62]、科学的、技術的 [63]、詩的[64]、美学的 (マルハナバチの羽音で満たされた春)、味覚的 (例えば蜂蜜を使った伝統的なケーキを保ち続けること)、そして文化的な価値がある。

世界の花粉媒介者数の減少とハチの不調

世界中で長期的な野生のハチの個体数減少が観察されている [47,65-70]。過去数十年以上、世界的にハチの不調の増加傾向やコロニーの消滅現象が現れている [71-77]。世界中の多くの場所でミツバチのコロニーの冬季の大量死多く発生している[72-75]。ネオニコチノイド系農薬が初めて使われた時、ハチが巣に帰って来ない、混乱している、地面に小さなグループで集まっている、異常な給餌行動、春に大量にハチがいなくなる、女王蜂がいなくなる、病気にかかりやすくなる、コロニーが消滅してしまうなど、養蜂家は様々な異なる不調や兆候を説明しはじめていた [38,40-43,77] 。これらの個々の兆候のどれもネオニコチノイド農薬の効果に特異的なものではなく、その他の要因や他の農薬も同じような兆候をもたらし得るため、因果関係を特定することを困難にする。

科学的調査では冬季のコロニー消滅の増加は 1 つの原因だけは説明できないと思われる。蜂群崩壊につながる全てのウィルスや病原体が、１年をとおして健康なコロニーでも見つかった[78]。これらの感染性物質の存在にも関わらず健康なままのコロニーがあることは、蜂群崩壊は要因が組み合わさって起きるという説を支持している。 Farooqui [79]は蜂群崩壊症候群(CCD)の説明を調査するにあたり科学者による異なる仮説を分析した。この研究では相互に要因を強化してしまう方向性を指摘している。これらの中で、独立した科学的結果を踏まえると、ネオニコチノイド農薬に関するものに重点が置かれる[80-82]. この論文では花粉媒介者の個体数減少とハチの不調の出現におけるネオニコチノイド農薬の役割に関する知識の現状について分析する。

多数の暴露経路

ネオニコチノイド系農薬は１年の異なる時期に花を咲かせる広い範囲の農業用、園芸用の植物に使用許可されている[34,37,83,84]。ネオニコチ

ノイド系農薬の浸透性の特徴は、花粉、花蜜、溢液にも輸送していることを示唆している。難分解性と農薬処理された作物の周辺の野生の植物や木が汚染されている可能性[36]と地表水や地下水を通して畑からはるか遠いところまで運ばれる可能性[27]、汚染された水を吸い上げることによって野生の植物や作物が汚染される可能性は、花粉媒介昆虫は一年中、給餌地域において多数のネオニコチノイドに多数の経路でさらされていることを意味するが、これはとても低い用量でさらされている。

ミツバチのネオニコチノイド系農薬への暴露は経口、接触と吸入（エアロゾル）によって起きる。多くの暴露経路が考えられる[85]。ここで私たちは暴露経路を以下のように集約した。（a）残留物を含むエサの摂取（b）巣に使う材料(樹脂、ロウなど）（c）農薬を使用している間の散布やほこりが流れてくるものに直接接触（d）汚染された植物、土、水への接触（e）巣の冷却水の使用（f）汚染された空気の吸入。 巣を土の中に作るマルハナバチとその他の野生のハチは土の汚染が暴露経路として追加される。ハキリバチは切り取った葉のカケラから巣の個室を作るため葉の残留農薬に暴露する可能性がある。他にもたくさん考えられる暴露経路はあり、例えば、ネオニコチノイド系農薬で処理され、この農薬が残留した木材からハチの巣が作られる可能性もある。しかしながら、最もよく研究された暴露経路はエサによるものである。残留のあるエサは、自分で集めた加工していないエサ（花蜜、花粉、水、植物の葉や茎から出る蜜、花外蜜、溢液の雫、給餌地域にある様々な他の食べられる物質）と、巣の中で加工されるエサ（蜂蜜、蜂パン、ロイヤルゼリー、ろうなど）と、養蜂家により与えられるエサ（高果糖コーンシロップ、砂糖水、砂糖団子、蜂用キャンディー、花粉、大豆の花から作られる花粉代替物、その他の植物たんぱくサプリメントなど）に分けられる。

ネオニコチノイド系農薬が使用された作物が非常に多く、大規模に使用されているため、特定の場所と時間で受けた総合的な暴露に関して、場所的・時間的に可能な暴露経路および相対的にどれが重要性を持つのかも非常に多様である。さらにミツバチの給餌地域は巣から半径 9km にも拡大していて、どれとして同質な景観ではないので、さらに複雑である[86]。さらに、庭や公園に花がたくさんあるため、野生のハチが郊外を拠点とすることもある[87]。それゆえ、ハチは庭の花や野菜、鑑賞用の木、芝生などに広く使われる浸透性農薬にさらされるかもしれない。相違的に重要な暴露経路は給餌範囲や生物季節学（季節的におこる自然界の動植物が示す諸現象の時間的変化）、一日の飛行時間などが異なるため、ハチの種類によってさまざまである。例えば、コーンが植えられた地域のツツハナバチはミツバチと比べると溢液の雫の摂取が重要である。

ミツバチの様々な分類によっても暴露経路も暴露の程度も異なる [42]。例えば花粉をとってくるミツバチ（花蜜をとってくるハチとは別）は花粉を摂取することはなく、巣に持ってくるだけである。花粉は子育て係のハチが摂取し幼虫に与えるため、ネオニコチノイド系農薬とその代謝物の残留物にさらされることになる[88]。花蜜をとる係が集めた花蜜のネオニコチノイド系農薬と代謝物への暴露するのは、巣の環境によってその暴露源が様々である。さらに給餌係は給餌のために巣を出発する前に巣の中から蜂蜜をいくらか持ちだす。 巣から給餌場所の距離によって、飛行や給餌のエネルギーのために、ミツバチは巣から持ち出した花蜜または蜂蜜をたくさんあるいは少し消費する。そのため、給餌環境により

残留したネオニコチノイド系農薬をたくさん摂取したり少ししか摂取しなかったりする [42]。経口摂取は給餌係のミツバチ、冬のミツバチ、幼虫で最も多いと予測される [85].

花粉に接触する観点からか、あるいは必要があれば花蜜への接触や消費の可能性の観点から考えると、コロニーの中の異なる係により汚染されたエサに実際にどれだけ暴露されているかはほとんど知られていない。野生のハチに関してはフィールドでの暴露に関してほとんどデータがない。野生のハチがフィールドで消費する量も計測されていない。EFSA はマルハナバチの働き蜂、女王蜂、幼虫と、孤立性ハチの大人のメスと幼虫は経口による残留摂取が最も多い可能性があると予測した [85]。

2002 年、フランスのミツバチに集められた花粉サンプルのうち 69%にイミダクロプリドおよびその代謝物が含まれていた[89]。5 箇所を組織的に 3 年間サンプリングしたところ、花粉の 40.5%、蜂蜜の 21.8%でイミダクロプリドが見つかった[90,91]。公認団体のデータによると、ネオニコチノイド処理された作物の花蜜及び花粉にあるネオニコチノイド残留物は、花蜜では検出制限値(0.3μg kg−1)以下から、高い場合は油用の菜種の花密で検出された 5.4 $\mu g\ kg^{-1}$、花粉だと検出制限値(0.3$\mu g\ kg^{-1}$)以下から、高いとアルファルファの花粉から検出された 51 $\mu g\ kg^{-1}$ のチアメトキサンに相当する量の間であると推定れている[85]。しかし、最近のレビューではさらに幅広い範囲が報告されている:花粉ではイミダクロプリドで>0.2 から 912 $\mu g\ kg^{-1}$、チアクロプリドで<1.0 から 115 $\mu g\ kg^{-1}$ である[92]。アメリカのラベルに記されている割合でかぼちゃに使用されたイミダクロプリド、ジノテフラン、チアメトキサン、そしてそれらの代謝物の残留物は、平均で花粉の 122 $\mu g\ kg^{-1}$ から花蜜の 17.6 $\mu g\ kg^{-1}$ にまでのぼる[93]。ネオニコチノイド処理された種から発芽した植物の葉から溢液現象(いつえき)により出た水滴からイミダクロプリド 346 $mg\ l^{-1}$ 、チアメトキサン 146 $mg\ l^{-1}$ 、そしてクロチアニジン 102 $mg\ l^{-1}$ が見つかった[84,94]。メロンにおいては、ラベルに記載されている容量の一番高い量を土壌に使用した3日後の溢液から 4.1 $mg\ l^{-1}$ のイミダクロプリドが見つかった[95]。アメリカ全土で行った蜜蝋、花粉、ハチにおける殺虫剤の残留物についての調査では、2007 年から 2008 年のシーズン中の花粉とミツバチにおいて、花粉で高い値のネオニコチノイドが見つかり([92]に含まれる)、イミダクロプリドが多いと 13.6 $\mu g\ kg^{-1}$ も蜜蝋でも見つかった[96]。スペインでは、果樹園の近くにある養蜂場の蜜蝋のサンプルからネオニコチノイドが見つかった:30 サンプル中 11 サンプルで 11 $\mu g\ kg^{-1}$ (アセタミプリド)から 153 $\mu g\ kg^{-1}$ (チアクロプリド)の範囲で陽性となった [97]。

葉や茎から出る蜜にネオニコチノイドが含まれているかについては良く知られていない。アブラムシと蜂の寿命の違いを考慮すると、樹液に含まれている濃度がアブラムシを殺すには低すぎたとしても、それが葉や茎から出る蜜へ輸送され、蜂や蜂のコロニーにとって亜致死および慢性毒性による致死を引き起こすことも出来る。

致死および亜致死暴露による急性および慢性的な影響

農薬は蜂に 4 種類の影響を及ぼす:急性致死、急性亜致死、慢性致死、慢性亜致死である。

急性毒性は短縮して「LD50(48h)」と記され、48 時間で暴露された半数のミツバチが死ぬ致死量(lethal dose: LD)として表現される。ネオニコチノイドは経口でも接触によってもミツバチにとって非常に毒性が高い(ng/蜂の範囲)[98]。これら

はこれまでにテストされた数種類のマルハナバチ類(*Bombus species*)、ツツハナバチ(*Osmia lignaria*)、アルファルファハキリバチ(*Megachile rotundata*)など他の種類の蜂にも非常に急性毒性が高い[99-102]。ツツハナバチはハナバチの一種(*B. impatiens*)よりもクロチアニジンおよびイミダクロプリドに感受性が高く、アルファルファハキリバチはさらに感受性が高い[100]。セミフィールド条件で行ったインドのミツバチ(*Apis cerana indica*)の急性毒性実験では、クロチアニジンが一番高い毒性を示し、続いてイミダクロプリド、チアメトキサンだった[103]。

春にハチの大量死が、ネオニコチノイドでコーティングされたとうもろこしの種をまいている近くで、しかも種まきの最中に起こるというのは、空気圧送式種まき機によって巻き上げられる埃に接触することによる急性毒性であるのはひとつひとつそこには証明しうるなんらかの結びつきがある。採餌をしに行ったのが隣接する森(蜜を提供する)であろうと、近くの花畑であろうと[104-109]。こうしたコロニーの消失はイタリア、ドイツ、オーストリア、そしてスロベニアでもとうもろこしの種まきの時期に報告されている[110,111,104]。こうした事件に反応し、規制により種をコーティングする技術が改善され、種まきの技術の改善もヨーロッパ全土で義務化された[112]。採掘機にエアディフレクタが設置され、種のコーティング技術が改善されようと、排出物はいまだに多く、排塵はハチにとって急性毒性をもつ[105,109,111,113-115]。空気中に微粒子状で散乱されたネオニコチノイドの急性毒性効果は高湿度の環境でより強まるようで、致死率を加速させる[105]。ミツバチは、自身の体に付着した毒性の粉塵粒子を巣に持ち帰る[106]。晴れて暖かい日もまた活性物質の散乱を手助けするようだ[35]。

慢性暴露による致死的影響とは、長期的な暴露後に起こるミツバチの死のことである。急性の致死的影響と違い慢性の致死的影響にはこれを計測する標準化された手法がない。したがって、殺虫剤のリスクアセスメントではこれまで 3 つの方法で表現されてきた。LD50、つまり暴露した50%のミツバチが死んでしまう濃度(10 日間の間に、ということが多いが違う日数のこともある)、NOEC (No Observed Effect Concentration)、つまりイミダクロプリドが観察できるような影響を与えない最高濃度、そして LOEC (Lowest Observed Effect Concentration) 、つまりイミダクロプリドが観察できるような影響を与える最低濃度だ。しかしネオニコチノイドやその神経毒性を持つ代謝物の致死毒性は暴露時間が長引くに連れ、急性毒性に比べ 100,000 倍も増えることがある[10]。Maxim and Van der Sluijs [40,42]によって詳細にわたり討論されているこの発見は、論議を巻き起こしている。しかし、暴露時間がネオニコチノイドの毒性を増幅させているという重要な発見はその後の発見と一致している。イミダクロプリドを摂食したマルハナバチのマイクロコロニーも同じ現象を示した[102]。10 分の 1 の濃度の毒素を摂食したマルハナバチのマイクロコロニーでは 100%の死に至るのに 2 倍の時間がかかった。100 分の 1 の濃度の毒素の場合 100%の死に至るのに約 4 倍の時間がかかった。計測可能な寿命の短縮は、(挿入された)慢性的な中毒時間がマルハナバチの働き蜂の自然な寿命よりも長くなる量を投与されたときに初めて止まった。これはネオニコチノイドの 10 日間の慢性毒性テストはハチにとって短すぎることを示唆している。実際、LC50 の 10 分の 1 の量のチアメトキサンを投与されたミツバチの 41.2%で寿命の短縮が起きている[116]。ネオニコチノイドの慢性毒性に関して、最近の研究では 10 日間の

LD50 よりも死んだ個体が 50%になるまでの時間のほうがより的確な可能性が示されている[117-122]。Log(一日量)と log(50%死までの時間)の間には線形関係がある[118,120,121]。ミツバチのコロニーを使った実験では類似した期の慢性影響が見つかっている。主に 20 $\mu g\ kg^{-1}$ のイミダクロプリドを含む食べ物に暴露した場合、14 から 23 週間で [123]、1 $mg\ kg^{-1}$ のジノテフランおよび 400 $\mu g\ kg^{-1}$ のクロチアニジンの場合 80 から 120 日で 25 から 100%のコロニーが消滅する[76]。これらの研究に使用された濃度は現在報告されているフィールドの濃度範囲のかなり高い濃度であることを述べておく。しかしこうしたデータもまばらで、いくつかの作物に限られており、このような濃度はフィールドにおいて一般的なのか否かについてはまだ結論付けることはできない。

ネオニコチノイドは低濃度で亜致死の影響が起こることがある。亜致死の影響はミツバチの行動や生理学的(免疫系など)な変化を巻き込む。直接個体およびコロニーの死に関与することはないが、時間と共に死に至らせたり、コロニーを過敏に(例えば病気になりやすくなるなど)したりして、コロニーの消滅に関与する可能性がある。例えば、記憶や方向感覚、身体的な不具合を抱えた個体は巣に戻ることが出来なくなり、空腹や寒さで死に至るかもしれない。これは通常急性致死にフォーカスを宛てる殺虫剤の標準的なテストでは検出されない。急性と慢性の亜致死性影響には違いがある。急性の亜致死の影響は特定の物質に 1 回暴露された状態(摂食および接触)で、その後特定の期間観察(研究室により数分から 4 日間と様々)され評価する。慢性亜致死の影響に関しては、長期間(例えば 24 時間から 10 日間)の間、ネオニコチノイドをミツバチに一回以上暴露させて評価する。急性および慢性亜致死影響共に NOEC もしくは LOEC またはその両方によって表現される[42]。

膨大なレビューの中で、Desneux et al.は、ネオニコチノイドの亜致死影響は神経生理学、幼虫の成長、脱皮、成虫の寿命、免疫、排泄、男女率、運動、ナビゲーションとオリエンテーション、給餌行動、産卵行動、記憶に影響を与えることを見つけた[124]。これらの影響は全ての花粉媒介者に関して報告され、全てにおいてポリネーターのコロニー、種族、コミュニティーレベルのインパクトを起こす可能性がある。

フィールドの現実的な濃度(1 $\mu g\ l^{-1}$)のイミダクロプリドは授粉媒介する甲虫を遠ざけ、検出制限よりもずいぶん下の濃度(0.01 $\mu g\ l^{-1}$)で授粉媒介するハエも遠ざける[125]。これはイミダクロプリド汚染は、汚染された自然と共に農地も崩壊させる可能性があることを示唆する。ミツバチにとって、イミダクロプリドはフィールドの現実的な濃度では回避作用を持たず、500 $\mu g\ l^{-1}$ になってようやく回避する[126]。植物保護薬のいくつかには、ネオニコチノイドと共にハチ除けの物質が混ぜられている。しかしネオニコチノイドは回避物質よりも長持ちし、また組織的な物性も違う。また、ハチが汚染された花を避けるようになったところで、ハチによる授粉が行われなくなってしまうのだ。

亜致死濃度のネオニコチノイドはミツバチの嗅覚記憶や学習能力[127-130]、オリエンテーションや採餌行動[131]を不能にさせる。亜致死暴露の飛行行動やナビゲーション能力におけるインパクトに関しては帰巣飛行試験により示されている[82,126,132,133]。非常に低濃度(0.05 $\mu g\ kg^{-1}$)のイミダクロプリドに暴露されたミツバチは、はじめは飛行距離が少し長くなる。しかし濃度が上がるに釣れ 0.5 $\mu g\ kg-1$ のイミダクロプリドから距離および個体間の交流時間が短くなり、食

べ物のある領域に到着するまでの時間は濃度と共に長くなる[134]。イミダクロプリドは 0.21 and 2.16 ng bee^{-1} でミツバチのダンスや糖反応を崩壊させる[135]。

もしミツバチの幼虫が理想以下の温度（蜂の成虫個体数が少ないことにより、温度を保てない）で育てられると、新しく成長した働き蜂は寿命の短縮と殺虫剤に対する過敏という特徴が出る（ハチレベルの影響）[136]。これはまたさらに、幼虫を育てるのに理想的な温度を保つのに必用な成虫個体数を満たせないことにつながり、コロニーは慢性的に弱体化して行き、最終的に崩壊してしまう（コロニーレベルの影響）。

亜致死影響は、マルハナバチ（*Bombus terrestris*）が、たとえその距離が短くても食べ物を集めに行かなくてはならない場合により頻繁に、そして低濃度で検出されるようだ。フィールドの現実的濃度での、巣で食べ物を与えられたマルハナバチのマイクロコロニーに対するイミダクロプリドの影響はないが、働き蜂がたった20cmのチューブを食べ物をとりに行くために歩くと、平均亜致死影響濃度（(EC50) が 3.7 μg kg^{-1} で採餌に有意な亜致死の影響が観られた[102]。温室の中で、餌が巣から3mはなれたところにあるマルハナバチの一種（queenright bumblebee）のコロニーでは、イミダクロプリドは 20 μg kg^{-1} で働き蜂の死に顕著な影響を及ぼし、蜂は餌箱で死んでしまう。顕著な致死率への影響は 10 μg kg-1 でもみられたが、2 μg kg-1 ではみられなかった[102]。マルハナバチはイミダクロプリドに対し、シロップに入った 1 μg l-1 から濃度依存的に亜致死反応（摂食率の減少）を示したが、ミツバチには影響がないようであった[137]。

フィールドに応答する濃度のイミダクロプリドは単独もしくはλ-シハロトリン（λ-cyhalothrin）との混合でマルハナバチコロニーの花粉採取効率を不能にした[138]。コロニーの花粉需要を満たすために、より多くの働き蜂が幼虫の世話ではなく採餌に借り出された。これは幼虫の成長に影響を及ぼし、働き蜂の減少という結果をもたらした[138]。実験室でマルハナバチのコロニーがフィールドの現実濃度のイミダクロプリド(花密で 0.7 μg kg^{-1} 、花粉で 6 μg kg^{-1})に2週間暴露された。その後、フィールドに戻され、6 週間自然な状況下におかれると、農薬を浴びたコロニーでは女王蜂が 85%少なくなり、成長率が顕著に下がった[81]。イミダクロプリドのマルハナバチの生殖に及ぼす影響は 1 μg l^{-1} のようなフィールドでも十分ありえる濃度でも起こる[139]。

イミダクロプリドのような殺虫剤は幼虫の世話をするミツバチの下咽頭腺の組織を退化させることも示されており[140-142]、巣からフィールドの行動シフトを誘発する。もともと針の無いキオビオオハリナシバチ（*Melipona quadrifasciata anthidioides*）では、イミダクロプリドは学習に関わるキノコ体を不能にする[143]。イミダクロプリドとクロチアニジンはミツバチの脳内の強力な神経修飾物質であることが示されており、ミツバチのキノコ体の神経細胞不活性化を引き起こし、採餌中や巣で暴露される濃度で認知や行動に影響を及ぼす[8]。亜致死量のイミダクロプリドは、排泄や浸透圧調整に関わるマルピーギ管の細胞毒性作用を持つことが示されている[144]。チアメトキサンへの暴露もハチの脳や中腸の形態不全を引き起こすとされる[116]。

ネオニコチノイドの残留物への暴露はミツバチの成長を特に最初の段階（4 日目から 8 日目）で遅らせる[145]。これは寄生ダニ（ヘギイタダニ科 Varroa destructor）のコロニー内での成長に有利な環境を与える。同じように、幼虫のころ暴露された成虫のハチの寿命は他より短い。

短期から中期の個体もしくは年齢集団への亜

致死影響はコロニーレベルでは長期的な影響を引き起こし、暴露後数週間から数ヶ月続き、ミツバチのコロニーの個体数減少やマルハナバチの女王の製造にまで及ぶ[76,81,123,138]。最近知られるようになったように、マーケティング会社が行ったネオニコチノイドのフィールドテストは、亜致死および長期的なコロニーレベルの影響を見るために作られてはおらず、実験的な暴露のコロニーの性能などについての観察は十分に長くは行われていない[85]。既存のフィールド実験においての主な弱点はコロニーのサイズが小さいこと、巣と処理されたフィールドとの距離が短いこと、そしてとてもテストフィールドの表面がとても低いことだ。こうした弱点により、フィールド実験中のミツバチの実際の暴露に関して非常に不確実で、実際はこうしたフィールド実験で推定されたよりももっと少ないかもしれない[85]。

さらに、メタアナリシス[146]が示すように、欧米の許可が基準としているこれまでに報告されたフィールドテストでは、メタアナリシスから誘発された濃度依存的な関係から予想されるコロニー性能の低下を検出するのに必用な統計的件出力を欠いている。この目的には、こうした実験のデザインはまちがっており、各実験グループのコロニー数は少なすぎ、長期のコロニーレベルのインパクトをモニターするフォローアップの期間も、上記に述べたようなことを検出するには短か過ぎる。しかしながら、これらのフィールド実験が、ヨーロッパ安全委員会や各国で現在見られるマーケット許可のベースになっているのである。メタアナリシスはこれまでの 14 のフィールド実験のデータをあわせており、フィールドでの現実的な濃度での暴露では、イミダクロプリドは顕著な亜致死影響をおよぼし、そして許可されたレベルでの使用でも性能を損失させ、したがってミツバチのコロニーを弱体化させる[146]。

さらにこれらのフィールド実験の制限は、巣から半径9キロにも及ぶミツバチの採餌領域の環境状況が非常に様々で、再現に制限があるということである。特定のフィールドで行われた観察は実際の条件で起こる様々な影響の代表とは必ずしもなりえない。コントロールできない様々な条件により（他のストレス要因、土壌構成、天気、蜂にとって魅力的な植物のコンビネーションなど、現在のフィールド実験に関しては、その実験が行われた特定のシチュエーションにおいてのみの情報を提供している。

フィールド実験の課題は、英国環境食糧省（Department for Environment, Food and Rural Affairs: DEFRA）傘下にある英国食料環境研究庁（Food and Environment Research Agency: FERA）が行い、フィールド実験が巻き起こした論争が明らかにしている。この実験は、フィールドで現実的なイミダクロプリドの濃度を短期間マルハナバチに暴露させると、長期間で 85%の女王蜂減少が起こると報じた Science の記事に対するものとして行われた[81]。農薬処理されない、クロチアニジン処理された、そしてイミダクロプリド処理された種から作物が育てられている3つの場所に20のマルハナバチのコロニーが曝された。そして FERA は農薬のレベルと虫への被害には「明確な関係は見られない」と結論付けたのだ。

[FERA: URL: http://www.fera.defra.gov.uk/scienceResearch/scienceCapabilities/chemicalsEnvironment/documents/reportPS2371Mar13.pdf].

しかし、コントロールとしたコロニーも実は試験対象となった農薬に汚染されていたのだ。さらに、3つのうち2つのハチの集団から、実験には使用されていないチアメトキサムが検出された[147]。ミツバチが集めてくる花粉のネオニコチノイド残

留物を計測する主要な研究ではすでに、ネオニコチノイドは年間を通してどんな領域からも見つかり、しかも種まきや花の咲いている季節に限らないことを明確にしている[89,91,96]。今日使用されているスケールでは、ハチがネオニコチノイドに曝されることのない場所をコントロールとして見つけるのは非常に困難だろう。

フィールド実験の結果の信憑性に大きな制限があるため、条件がコントロールされたラボで再現可能なリスクアセスメントの実験に重点を置き、環境下の濃度と影響の出ない濃度の比率を重要なリスク指標としていく方が良いだろう[40,42]。どのようにして何が、コロニーを弱体化させるハチ個体への知られている亜致死影響を検証するモデリングとリンクさせることも可能だろう[148]。

ミツバチの生物学のキーとしては、コロニーが「超生物（superorganism）」として行動することだ[149]。コロニーの中では、コロニーを保つために必用な様々なタスクを行うメンバーを確保するため、十分なメンバーが不可欠であり、各個体がどれだけ単独でうまくタスクを行うことが出来るかではないのだ。冬と夏では違うが、約 1 万から 6 万のミツバチが共同単位としてのコロニー機能を形成し、生物内恒常性機能、食物保管、巣の衛生管理、巣の守備、幼虫の世話などを保っている。したがって亜致死影響は、どのタスクを行っている個体が何体影響されるかがコロニー機能全体に影響を与える。単純化された理論モデリングのアプローチでは、コロニーの崩壊はミツバチの個体数の力学のプリンシパルを観察すれば理解できるのかもしれない[150]。コロニーのシミュレーションモデルは個体数の急速な減少により巣の崩壊が免れない採餌個体の死亡率の臨界閾値を予想している。採餌個体の高い死亡率は巣にいる個体を通常より若年の段階で採餌集団に引き込み、コロニーの崩壊を加速させる[150]。

相乗的影響：農薬-農薬＆農薬-感染要因

単独のストレッサーの効果を加算するよりも、併用されたときに起こる効果が大きい場合に相乗効果が起こる。ネオニコチノイドが特定の殺菌剤（プロクロラズのようなアゾールやアニリドのようなメタラキシルなど）やシトクローム P450 解毒酵素をブロックするようなほかの農薬と併用されると、組み合わせにもよるが、1.52 から 1,141 倍も毒性が高まる[151,152]。この中でも一番高い相乗効果を持つものとして、トリフルミゾールとチアクロプリドの組み合わせが挙げられ、ミツバチにとっては 1,141 倍も急性毒性が高まる[151]。この相乗効果は農薬会社の特許対象となっている[152,153]。

相乗効果はネオニコチノイドと感染要因でも見られる。亜致死量のネオニコチノイドへの長期間の暴露は、巣全体がノゼマ原虫（*Nosema ceranae*）などの寄生虫に感染しやすくなる[39, 154-156]。これは免疫系の変化および単独および個体間での毛づくろいが出来なくなること個体レベルおよび巣内の衛生状態が劣化することで説明ができ、病原体がハチを感染しやすくする。亜致死量のネオニコチノイド暴露によって毛づくろいが出来なくなり、虫と天敵のバランスが崩されるのと同じメカニズムはしばしば害虫管理として標的虫に使われることで知られている[157-161]。

まとめと展望

20 年も経たないうちにネオニコチノイドは世界でもっともよく使われる類の殺虫剤となった。120 カ国以上で 1000 以上の様々な作物や用途で使用され、現在少なくとも世界中の殺虫剤市場の 4 分の 1 を占めている。花粉媒介者にとっては、ほ

とんどの作物および野生の花の花粉や花蜜がネオニコチノイドを様々な濃度で含有するというように、農薬の眺望をすっかり変えてしまった。ほとんどのネオニコチノイド系農薬は土壌や水、堆積物に長く残り、くり返し使われることで土壌に蓄積していく。ネオニコチノイドによる表面水のひどい汚染も一般的だ。この農薬の組織的な作用の仕方は、師管や木部を通して花粉や花蜜までも輸送されることを意味している。幅広い用途、作物や野生の植物からも取り込まれる可能性のある土壌内や水中の残留は、ネオニコチノイドをポリネーターに対して年間を通して亜致死濃度で生体利用可能にしている。こうしてネオニコチノイドは蜂の巣に頻繁に現れるようになるのである。ネオニコチノイドはミツバチや野生の花粉媒介者にとって非常に神経毒性が強い。中枢神経を囲むイオン不浸透性のバリア（血液関門: blood brain barrier: BBB）を越えることができ、ハチの中枢神経内で nAChR に強力な結合ができるというのがこの農薬の独特な亜致死毒性の原因である。ネオニコチノイドの毒性は暴露時間でより強化される。いくつかの研究では LD50 よりはるかに低い量では、非単調な量依存的な曲線が示されている[162]。春に起こる急性毒性によるハチの大量死はドイツ、イタリア、スロベニア、そしてフランスでネオニコチノイド処理されたとうもろこしの種を植えている最中に起こっている。採餌中のハチが種植え中のトウモロコシ畑のそばを通ると、種まき機によって巻き上げられる毒性の粉塵により急性致死量に暴露されることになる。

フィールドで現実的な暴露レベルでは、ネオニコチノイドは数多くの亜致死影響をミツバチやマルハナバチのコロニーに引き起こし、採餌の成功度を狂わせ、卵や幼虫の成長、記憶や学習を妨げ、中枢神経を損傷させ、病気にかかりやすくなり、巣の衛生状態が悪くなる。ネオニコチノイドはノゼマ原虫など（*Nosema ceranae*）の感染要因を相乗的に強化させ、他の農薬とも毒性を相乗させる。マルハナバチのコロニーが短期のフィールドで現実的な暴露を受けると、マルハナバチの女王蜂に長期的な影響がある（85%減少）というのは、世界的に見られるマルハナバチの減少に寄与している。他の野生のポリネーターへの毒性を検証している研究は数少ないが、現在あるデータでは他の野生のポリネーターとほぼ同じような毒性を示しているようである。ネオニコチノイドの世界的な生産は今でも増え続けている。花粉媒介昆虫が自然および農業の生態系に寄与する致命的な重要性を考慮すると、こうした虫たちは大切に保護されるべきである。したがって、ポリネーターの生態系への奉仕を持続するためには、ポリネーターに優しいネオニコチノイドに変わるものが即急に必用である。先日欧州委員会が行った蜂にとって魅力的な作物にイミダクロプリド、チアメトキサン、クロチアニジンを使用することを規制する決断は、そうした方向に向けたはじめの一歩といえる。

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Current Opinion in Environmental Sustainability

Neonicotinoids, bee disorders and the sustainability of pollinator services☆

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In less than 20 years, neonicotinoids have become the most widely used class of insecticides with a global market share of more than 25%. For pollinators, this has transformed the agrochemical landscape. These chemicals mimic the acetylcholine neurotransmitter and are highly neurotoxic to insects. Their systemic mode of action inside plants means phloemic and xylemic transport that results in translocation to pollen and nectar. Their wide application, persistence in soil and water and potential for uptake by succeeding crops and wild plants make neonicotinoids bioavailable to pollinators at sublethal concentrations for most of the year. This results in the frequent presence of neonicotinoids in honeybee hives. At field realistic doses, neonicotinoids cause a wide range of adverse sublethal effects in honeybee and bumblebee colonies, affecting colony performance through impairment of foraging success, brood and larval development, memory and learning, damage to the central nervous system, susceptibility to diseases, hive hygiene etc. Neonicotinoids exhibit a toxicity that can be amplified by various other agrochemicals and they synergistically reinforce infectious agents such as *Nosema ceranae* which together can produce colony collapse. The limited available data suggest that they are likely to exhibit similar toxicity to virtually all other wild insect pollinators. The worldwide production of neonicotinoids is still increasing. Therefore a transition to pollinator-friendly alternatives to neonicotinoids is urgently needed for the sake of the sustainability of pollinator ecosystem services.

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Introduction

The introduction to the market in the early 1990s of imidacloprid and thiacloprid opened the neonicotinoid era of insect pest control [1]. Acting systemically, this new class of neurotoxic insecticides is taken up by plants, primarily through the roots, and translocates to all parts of the plant through xylemic and phloemic transport [2]. This systemic property combined with very high toxicity to insects enabled formulating neonicotinoids for soil treatment and seed coating with typical doses from 10 to 200 g ha^{-1} high enough to provide long lasting protection of the whole plant from pest insects.

Neonicotinoids interact with the nicotinic acetylcholine receptors (nAChRs) of the insect central nervous system. They act mainly agonistically on nAChRs on the postsynaptic membrane, mimicking the natural neurotransmitter acetylcholine by binding with high affinity [3–5,6••,7•,8••]. This induces a neuronal hyper-excitation, which can lead to the insect's death within minutes [6,9]. Some of the major metabolites of neonicotinoids are equally neurotoxic, acting on the same receptors [10–12] thereby prolonging the effectiveness as systemic insecticide. The nAChR binding sites in the vertebrate nervous system are different from those in insects, and in general they have lower numbers of nicotinic receptors with high affinity to neonicotinoids, which are the reasons that neonicotinoids show selective toxicity for insects over vertebrates [9,13].

The main neonicotinoids presently on the market are imidacloprid, thiamethoxam, clothianidin, thiacloprid, dinotefuran, acetamiprid, nitenpyram and sulfoxaflor [12,14,15]. Since their introduction, neonicotinoids have grown to become the most widely used and fastest

growing class of insecticides with a 2010 global market share of 26% of the insecticide market [16] and imidacloprid the second most widely used (2008) agrochemical in the world [17]. The worldwide production of neonicotinoids is still increasing [18]. Large-scale use in Europe and US started around 2004. Neonicotinoids are nowadays authorised in more than 120 countries for more than 1000 uses [19] for the treatments of a wide range of plants including potato, rice, maize, sugar beets, cereals, oil rapeseed, sunflower, fruit, vegetables, soy, ornamental plants, tree nursery, seeds for export, and cotton.

When used as a seed coating, only 1.6–20% of the amount of active substance applied actually enters the crop to protect it [20], and the remaining 80–98.4% pollutes the environment without any intended action to plant pests. Diffusion and transformation of pesticides in the environment lead to various environmental concentrations and bioavailability, all strongly dependent on the properties of the substance [21]. Because of their high leaching potential, neonicotinoids tend to contaminate surface water and ground water [22–25]. Owing to sorption to organic matter in soil and sediments [24,26], the equilibrium partitioning over soil and water varies with soil type and is typically 1:3 ($\log P = 0.57$) [25]. In countries where monitoring data are available, high levels of neonicotinoid pollution in surface water have been reported [27–30]. In the Netherlands, 45% of 9037 water samples taken from 801 different locations in a nation-wide routine water quality monitoring scheme, over the period 1998 and 2003–2009, exceeded the 13 ng l^{-1} imidacloprid water quality standard, the median concentration being 80 ng l^{-1} and the maximum concentration found being 320 μg l^{-1}, which is acutely toxic to honeybees [27]. In the US, neonicotinoids were also found in surface water. In 108 water samples collected in 2005 from playa wetlands on the Southern High Plains, thiamethoxam was found at an average concentration of 3.6 μg l^{-1} and acetamiprid at 2.2 μg l^{-1} [30].

Neonicotinoids and their metabolites are highly persistent in soil, aquatic sediments and water. To give an example: Six years after a single soil drench application of imidacloprid, residue levels up to 19 μg kg^{-1} could be recovered in *Rhododendron* shrub blossoms [31]. Clothianidin has a half-life in soil between 148–6900 days [32], and imidacloprid 40–997 days [33]. Consequently, neonicotinoids exhibit a potential for accumulation in soil following repeated applications [23] and can be taken up by succeeding crops up to at least two years after application [34]. Imidacloprid has been detected in 97% of 33 soil samples from untreated fields on which treated corn seeds were used 1 or 2 years before the sampling [34]. Concentrations in these soil samples ranged from 1.2 to 22 μg kg^{-1} [34]. Several studies recovered neonicotinoids in wild flowers near treated fields [35,36••]. However, it remains a knowledge gap to what extent the presence in wild flowers results from systemic uptake from polluted soil and water or from direct contamination of the flowers by contaminated dust from seed drilling.

At their introduction, neonicotinoids were assumed to be more efficient than the organophosphates and carbamates that they replaced [37]. As a seed treatment, they could be used in much lower quantities and they promised to be less polluting to the environment. It is however not the quantity that is relevant but the potency to cause harm, which results from toxicity, persistence and bioavailability to non-target species. Indeed, soon after the introduction of neonicotinoids, exposure to its residues in pollen, nectar, sowing dust etc., of non-target pollinating insects became clear. This led to various harmful effects [10,37,38,39••,40,41,42••,43••].

Ecosystem services of pollinators

Amongst the wide diversity of pollinating species [44], bees are the most important. Although bee research mostly focuses on the domesticated *Apis mellifera*, over 25,000 different bee species have been identified (FAO: Pollination; URL: http://www.fao.org/agriculture/crops/core-themes/theme/biodiversity/pollination/en/). Bees provide a vital ecosystem service, playing a key role in the maintenance of biodiversity and in food and fibre production [45–47,48••,49–51]. Pollination comprises an integrated system of interactions that links earth's vegetation, wildlife and human welfare [52]. Of all flowering plants on earth, 87.5% benefits from animal pollination [53]. Globally, 87 of the leading food crops (accounting for 35% of the world food production volume) depend on animal pollination [45]. Pollinator mediated crops are of key importance in providing essential nutrients in the human food supply [54•]. The history of apiculture goes back to pre-agricultural times [55,56] and later co-developed with agriculture [57,58]. In addition, wild bees deliver a substantial and often unappreciated portion of pollination services to agriculture and wildflowers [59,60]. Bees and apiary products have a pharmacological [61,62], scientific and technological [63], poetic [64], aesthetic (springs filled with buzzing bumblebees) culinary (e.g., keeping alive traditional cuisine of patisseries with honey) and cultural value.

Global pollinator decline and emerging bee disorders

Long-term declines have been observed in wild bee populations around the world [47,65–70]. Over the past decades, a global trend of increasing honeybee disorders and colony losses has emerged [71–77]. Winter mortality of entire honeybee colonies has risen in many parts of the world [72•,73,74,75•]. When neonicotinoids were first used, beekeepers started describing different disorders and signs ranging from: bees not returning to the hive, disoriented bees, bees gathered close together in small groups on the ground, abnormal foraging behaviour, the

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occurrence of massive bee losses in spring, queen losses, increased sensitivity to diseases and colony disappearance [38,40–43,77]. None of these individual signs is a unique effect of neonicotinoids, other causal factors or other agrochemicals could produce similar signs, which complicates the establishment of a causal link.

Scientific research appears to indicate no single cause explaining the increase in winter colony losses. All viruses and other pathogens that have been linked to colony collapse have been found to be present year-round also in healthy colonies [78]. That colonies remain healthy despite the presence of these infectious agents, supports the theory that colony collapse may be caused by factors working in combination. Farooqui [79•] has analysed the different hypotheses provided by science when searching for an explanation of Colony Collapse Disorder (CCD). Research points in the direction of a combination of reciprocally enhancing causes. Among those, the advance of neonicotinoid insecticides has gained more weight in light of the latest independent scientific results [80,81••,82••]. In the present article, we synthesise the state of knowledge on the role of neonicotinoids in pollinator decline and emerging bee disorders.

Multiple ways of exposure

Neonicotinoids are authorised for a wide range of agricultural and horticultural plants that flower at different times of the year. The systemic properties of neonicotinoids imply translocation to pollen, nectar, and guttation droplets [34,37,83,84]. The persistency and potential contamination of wild plants and trees surrounding the treated crops [36] and the possibility for travelling far outside the fields via surface and ground water [27] and the potential to contaminate wild plants and crops that take up polluted water, means that pollinating insects are likely to be exposed for much of the year to multiple sources of multiple neonicotinoids in their foraging area, but often at very low doses.

Honeybees' exposure to neonicotinoids can occur through ingestion, contact and inhalation (aerosols). Many possible exposure pathways can exist [85•]. Here, we aggregate exposure pathways into: first, intake of food that contain residues; second, nesting material (resin, wax etc.); third, direct contact with spray drift and dust drift during application; fourth, contact with contaminated plants, soil, water; fifth, use of cooling water in the hive; and sixth, inhalation of contaminated air. For bumble bees and other wild bees that nest in soil, contact with contaminated soil is an additional pathway of concern. Leafcutter bees use cut leaf fragments to form nest cells and can thus be exposed to residues in leaves. There are many other conceivable exposure routes, for instance, a bee hive could have been made from timber from trees treated with neonicotinoids and may thus contain residues. However, the best researched exposure pathway is via intake of food. Food with residues can be subdivided into self-collected raw food (nectar, pollen, water, honeydew, extrafloral nectar, guttation droplets, various other edible substances available in the foraging area etc.), in-hive processed food (honey, beebread, royal jelly, wax etc.), and food supplied by bee keepers (high fructose corn syrup, sugar water, sugar dough, bee candy, pollen, pollen substitutes based on soybean flower and other vegetable protein supplements etc.).

Given the large numbers of crops in which neonicotinoids are used and the large scale of use, there is a huge variability in space and time for each possible exposure pathway as well as in their relative importance for the overall exposure at a given place and time. This is further complicated by the fact that the foraging area of a honeybee colony can extend to a radius of up to 9 km around the hive which is never a homogenous landscape [86]. Additionally, suburban areas have become a stronghold for some wild bee species due to the abundance of floral resources in gardens and parks [87]. Thus, bees may be exposed to systemic insecticides which are widely used on garden flowers, vegetables, ornamental trees, and lawns. The relative importance of exposure pathways will also vary according to bee species as they have different foraging ranges, phenologies, and flight times in a day. This can be exemplified by *Osmia* bees in corn growing areas for which intake of guttation droplets may be more important than for honeybees.

Different categories of honeybees could be exposed in different ways and to varying extents [42]. For example, pollen foragers (which differ from nectar foragers) do not consume pollen, merely bringing it to the hive. The pollen is consumed by nurse bees and to a lesser extent by larvae which are thus the ones that are exposed to residues of neonicotinoids and their metabolites [88]. The exposure of nectar foragers to residues of neonicotinoids and metabolites in the nectar they gather can vary depending on the resources available in the hive environment. In addition, foragers take some honey from the hive before they leave for foraging. Depending on the distance from the hive where they forage, the honeybees are obliged to consume more or less of the nectar/honey taken from the hive and/or of the nectar collected, for energy for flying and foraging. They can therefore ingest more or less neonicotinoid residues, depending on the foraging environment [42]. Oral uptake is estimated to be highest for forager honeybees, winter honeybees and larvae [85].

Little is known about the real exposure to contaminated food for different categories of honeybees in a colony, either in terms of contact with pollen or contact with, and possible consumption of, nectar if needed. For wild bees very few data exist on exposure in the field. The amount that wild bees actually consume in the field has not been

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measured. EFSA estimated that worker bees, queens and larvae of bumblebees and adult females and larvae of solitary bees are likely to have the highest oral uptake of residues [85].

In 2002, 69% of pollen samples collected by honeybees at various places in France contained residues of imidacloprid and its metabolites [89]. In a systematic sampling scheme covering 5 locations over 3 years, imidacloprid was found in 40.5% of the pollen samples and in 21.8% of the honey samples [90,91]. On the basis of data from authorisation authorities, neonicotinoid residues in nectar and pollen of treated crop plants are estimated to be in the range of below analytical detection limit (0.3 $\mu g\ kg^{-1}$) to 5.4 $\mu g\ kg^{-1}$ in nectar, the highest value corresponding to clothianidin in oilseed rape nectar, and a range of below detection limit (0.3 $\mu g\ kg^{-1}$) to 51 $\mu g\ kg^{-1}$ in pollen, the highest value corresponding to thiamethoxam in alfalfa pollen [85]. A recent review reports wider ranges for pollen: 0.2–912 $\mu g\ kg^{-1}$ for imidacloprid and 1.0–115 $\mu g\ kg^{-1}$ for thiacloprid [92]. Residues of imidacloprid, dinotefuran, and thiamethoxam plus metabolites in pumpkin treated with United States label rates reach average levels up to 122 $\mu g\ kg^{-1}$ in pollen and 17.6 $\mu g\ kg^{-1}$ in nectar [93]. Up to 346 $mg\ l^{-1}$ for imidacloprid and 146 $mg\ l^{-1}$ for thiamethoxam and 102 $mg\ l^{-1}$ clothianidin and have been found in guttation drops from leaves of plants germinated from neonicotinoid-coated seeds [84,94]. In melon, guttation levels up to 4.1 $mg\ l^{-1}$ imidacloprid were found 3 days after a top (US) label rate soil application [95]. In a US wide survey of pesticide residues in beeswax, pollen and honeybees during the 2007–2008 growing seasons, high levels of neonicotinoids were found in pollen (included in [92]) but imidacloprid was also found up to 13.6 $\mu g\ kg^{-1}$ in wax [96]. In Spain, neonicotinoids were found in beeswax samples from apiaries near fruit orchards: 11 out of 30 samples tested positive in ranges from 11 $\mu g\ kg^{-1}$ (acetamiprid) to 153 $\mu g\ kg^{-1}$ (thiacloprid) [97].

Little is known on the presence of neonicotinoids in honeydew. Given differences in life span of aphids and bees, concentrations in plant sap too low to kill aphids could translocate to honeydew and could still produce sublethal effects and chronic toxicity mortality in bees and bee colonies.

Acute and chronic effects of lethal and sublethal exposure

Pesticides can produce four types of effects on honeybees: lethal effects and sublethal effects from acute or chronic exposures.

Acute toxicity is expressed as the lethal dose (LD) at which 50% of the exposed honeybees die within 48 hours: abbreviated to 'LD50 (48 hours)'. Neonicotinoids are highly toxic (in the range of ng/bee) to honeybees [98], both when administered orally and by contact. They also have high acute toxicity to all other bee species so far tested, including various *Bombus* species, *Osmia lignaria* and *Megachile rotundata* [99–102]. *O. lignaria* is more sensitive to both clothianidin and imidacloprid than is *B. impatiens*, with *M. rotundata* more sensitive still [100]. In an acute toxicity test under semi field conditions on the Indian honeybee *Apis cerana indica*, clothianidin showed the highest toxicity, followed by imidacloprid and thiamethoxam [103].

For mass-dying of bees in spring nearby and during sowing of corn seeds coated with neonicotinoids there now is a one to one proven causal link with acute intoxication though contact with the dust cloud around the pneumatic sowing machines during foraging flights to adjacent forests (providing honeydew) or nearby flowering fields [104••,105–109]. Such mass colony losses during corn sowing have also been documented in Italy, Germany, Austria and Slovenia [110,111,104••]. In response to the incidents, the adherence of the seed coating has been improved owing to better regulations, and an improved sowing-technique has recently become compulsory throughout Europe, [112]. Despite the deployment of air deflectors in the drilling machines or improved seed coating techniques, emissions are still substantial and the dust cloud is still acutely toxic to bees [105,109,111,113–115]. Acute lethal effects of neonicotinoids dispersed as particulate matter in the air seem to be promoted by high environmental humidity which accelerates mortality [105]. Honeybees also bring the toxic dust particles they gather on their body into the hive [106]. Sunny and warm days also seem to favour the dispersal of active substances [35].

Lethal effects from chronic exposure refer to honeybee mortality that occurs after prolonged exposure. In contrast to acute lethal effects, there are no standardised protocols for measuring chronic lethal effects. Therefore, in traditional risk assessment of pesticides they are usually expressed in three ways: LD50: the dose at which 50% of the exposed honeybees die (often, but not always, within 10 days); NOEC (No Observed Effect Concentration): the highest concentration of imidacloprid producing no observed effect; and LOEC (Lowest Observed Effect Concentration): the lowest concentration of imidacloprid producing an observed effect. However, for neonicotinoids and its neurotoxic metabolites, lethal toxicity can increase up to 100,000 times compared to acute toxicity when the exposure is extended in time [10]. There has been some controversy on the findings of that study, which is discussed in detail by Maxim and Van der Sluijs [40,42]. However, the key finding that exposure time amplifies the toxicity of neonicotinoids is consistent with later findings. Micro-colonies of bumblebees fed with imidacloprid showed the same phenomenon [102]: at one tenth of the concentration of the toxin in feed, it took twice as long to produce 100% mortality in a

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bumblebee microcolony. At a 100 times lower dose, it took ca. four times longer to produce 100% mortality. The measurable shortening of the life span ceases to occur only when a dose was administered, for which the (extrapolated) chronic intoxication time would be longer than the natural life span of a worker bumblebee. This implies that the standard 10 day chronic toxicity test for bees is far too short for testing neonicotinoids. Indeed, honeybees fed with one tenth of the LC50 of thiamethoxam showed a 41.2% reduction of life span [116]. Recent studies have shown that chronic toxicity of neonicotinoids can more adequately be expressed by time to 50% mortality instead of by the 10 day LD50 [117–120,121•,122]. There is a linear relation between log daily dose and log time to 50% mortality [118,120,121•]. In experiments with honeybee colonies, similar long term chronic effects have indeed been found with typical times of 14–23 weeks to collapse 25–100% of the colonies exposed to imidacloprid-contaminated food at 20 $\mu g\ kg^{-1}$ [123] and 80–120 days for 1 $mg\ kg^{-1}$ dinotefuran and 400 $\mu g\ kg^{-1}$ clothianidin [76]. Note that these studies used concentrations that are on the high end of the currently reported ranges of concentrations found in the field. However, such data are sparse and limited to a few crops, so it cannot yet be concluded whether such concentrations are rare or common in the field.

At low concentrations of neonicotinoids, sublethal effects can occur. Sublethal effects involve modifications of honeybee behaviour and physiology (e.g., immune system). They do not directly cause the death of the individual or the collapse of the colony but may become lethal in time and/or may make the colony more sensitive (e.g., more prone to diseases), which may contribute to its collapse. For instance, an individual with memory, orientation or physiological impairments might fail to return to its hive, dying from hunger or cold. This would not be detected in standard pesticide tests, which focus on acute mortality. A distinction can be made between acute and chronic sublethal effects. Acute sublethal effects are assessed by exposing bees only once to the substance (by ingestion or by contact), and observing them for some time (variable from one laboratory to another, from several minutes to four days). Chronic sublethal effects are assessed by exposing honeybees more than once to neonicotinoids during an extended period of time (e.g., every 24 hours, for 10 days). Both acute and chronic sublethal effects are expressed as NOEC and/or LOEC (No or Lowest Observable Effect Concentration, respectively) [42].

In an extensive review Desneux *et al.* found that sublethal effects of neonicotinoids exist on neurophysiology, larval development, moulting, adult longevity, immunology, fecundity, sex ratio, mobility, navigation and orientation, feeding behaviour, oviposition behaviour, and learning [124]. All these effects have been reported for pollinators and all have the potential to produce colony level, population level and community level impacts on pollinators.

At field realistic concentrations (1 $\mu g\ l^{-1}$) imidacloprid repels pollinating beetles while at concentrations well below the analytical detection limit (0.01 $\mu g\ l^{-1}$) it repels pollinating flies [125]. This implies that imidacloprid pollution may disrupt pollination both in polluted nature and in agricultural lands. On honeybees, imidacloprid has no repelling effect at field realistic concentrations: it starts being repellent at 500 $\mu g\ l^{-1}$ [126]. In some plant protection formulations, neonicotinoids are mixed with bee repellents. However, the persistence of neonicotinoids exceeds that of the repellence and their systemic properties differ. Besides, if bees are effectively repelled and avoid the contaminated flowers, pollination is disrupted because plants are not visited by bees.

Sublethal doses of neonicotinoids impair the olfactory memory and learning capacity of honeybees [127,128,129•,130] and the orientation and foraging activity [131]. The impact of sublethal exposure on the flying behaviour and navigation capacity has been shown through homing flight tests [82,126,132,133]. Exposed to a very low concentration (0.05 $\mu g\ kg^{-1}$) imidacloprid honeybees show an initial slight increase in travel distance. However, with increasing concentration, starting at 0.5 $\mu g\ kg^{-1}$ imidacloprid decreases distance travelled and interaction time between bees, while time in the food zone increases with concentration [134•]. Imidacloprid disrupts honeybee waggle dancing and sucrose responsiveness at doses of 0.21 and 2.16 $ng\ bee^{-1}$ [135].

If honeybee brood is reared at suboptimal temperatures (the number of adult bees is not sufficient to maintain the optimal temperature level), the new workers will be characterised by reduced longevity and increased susceptibility to pesticides (bee-level effect) [136]. This will again result in a number of adult bees insufficient to maintain the brood at the optimal temperature, which may then lead to chronic colony weakening until collapse (colony-level effect).

Sublethal effects seem to be detected more frequently and at lower concentrations when bumblebees (*Bombus terrestris*) have to travel to gather food, even when the distances are tiny. No observable impacts of imidacloprid at field realistic concentrations on micro-colonies of *B. terrestris* provided with food in the nest were found, but when workers had to walk just 20 cm down a tube to gather food, they exhibited significant sublethal effects on foraging activity, with a median sublethal effect concentration (EC_{50}) of 3.7 $\mu g\ kg^{-1}$ [102]. In queenright bumblebee colonies foraging in a glasshouse where food was 3 m away from their nest, 20 $\mu g\ kg^{-1}$ of imidacloprid caused significant worker mortality, with bees dying at the feeder. Significant mortality was also observed at

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10 μg kg^{-1}, but not at 2 μg kg^{-1} [102]. Bumblebees exhibit concentration-dependent sublethal responses (declining feeding rate) to imidacloprid starting at 1 μg l^{-1} in syrup, while honeybees seemed unaffected [137].

Field-relevant concentrations of imidacloprid, used alone or in mixture with λ-cyhalothrin, were shown to impair pollen foraging efficiency in bumblebee colonies [138•]. In an attempt to fulfill colony needs for pollen, more workers were recruited to forage instead of taking care of brood. This seemed to affect brood development resulting in reduced worker production [138•]. Bumblebee colonies have been exposed to field realistic levels of imidacloprid (0.7 μg kg^{-1} in nectar, 6 μg kg^{-1} in pollen) for two weeks in the laboratory. When subsequently placed back in the field and allowed to develop naturally for the following six weeks, treated colonies showed an 85% reduction in queen production and a significantly reduced growth rate [81••]. Effects on bumblebee reproduction occur at imidacloprid concentrations as low as 1 μg l^{-1} [139•] which is highly field-realistic.

It has also been shown that pesticides like imidacloprid act on the hypopharyngeal glands of honeybee nurses by degenerating the tissues [140,141,142••], which induces a shift from nest to field activities. In the native stingless bee *Melipona quadrifasciata anthidioides*, imidacloprid causes impairment of the mushroom bodies which are involved in learning [143]. Imidacloprid and clothianidin have been shown to be potent neuromodulators of the honeybee brain, causing mushroom body neuronal inactivation in honeybees, which affect honeybee cognition and behaviour at concentrations that are encountered by foraging honeybees and within the hive [8]. Sublethal doses of imidacloprid were also found to have cytotoxic activity in the Malpighian tubules in honeybees that make up the excretory and osmoregulatory system [144]. Exposure to thiamethoxam has also been shown to result in morphological impairment of the bee brain and bee midgut [116].

Exposure to neonicotinoid residues leads to a delayed development of honeybee larvae, notably in the early stages (day 4 to day 8) [145]. This can favour the development of the *Varroa destructor* parasitic mite within the colony. Likewise, the life span of adult bees emerging from the exposed brood proved to be shorter.

Short-term and mid-term sublethal effects on individuals or age groups result in long-term effects at the colony level, which follow weeks to months after the exposure, such as honeybee colony depopulation and bumblebee colony queen production [76,81••,123,138•]. As it has recently been acknowledged, the field tests on which the marketing authorisation of the use of neonicotinoids is essentially based were not developed to detect sublethal nor long-term effects on the colony level, and the observation of the performances of colonies after experimental exposure do not last long enough [85]. Major weaknesses of existing field studies are the small size of the colonies, the very small distance between the hives and the treated field and the very low surface of the test field. As a consequence of these weaknesses, the real exposures of the honey bees during these field tests are highly uncertain and may in reality be much smaller than what has been assumed in these field studies. [85]

In addition, the meta-analysis [146•] demonstrates that field tests published until now on which European and North American authorizations are based, lack the statistical power required to detect the reduction in colony performance predicted from the dose–response relationship derived from that meta-analysis. For this purpose, the tests were wrongly designed, there were too few colonies in each test group, and the follow up time monitoring the long term colony level impacts were too short to detect many of the effects described above. Nonetheless, these field studies have been the basis for granting the present market authorizations by national and European safety agencies. The meta-analyses combined data from 14 previous studies, and subsequently demonstrated that, at exposure to field realistic doses, imidacloprid does have significant sublethal effects, even at authorised levels of use, impairs performance and thus weakens honeybee colonies [146•].

A further limitation of field studies is their limited reproducibility due to the high variability in environmental conditions in the foraging area of honeybees, which extends up to a 9 km radius around the hive. Observations made in a particular field experiment might not be representative of the range of effects that could occur in real conditions. Owing to the large variability of factors that cannot be controlled (e.g. other stressors, soil structure, climate, combination of plants attractive to bees etc.), current field experiments only give information about the particular situation in which they were done.

The challenges of field studies became also clear in the debates over the highly contested field study recently conducted by the Food and Environment Research Agency (FERA) which resorts under the UK Department for Environment, Food and Rural Affairs (DEFRA). This study was set up in response to the *Science* publication that showed that a short term exposure of bumblebees to field realistic imidacloprid concentrations causes a long term 85% reduction in queen production [81••]. At three sites 20 bumblebee colonies were exposed to crops grown from untreated, clothianidin-treated or imidacloprid-treated seeds. The agency concluded that 'no clear consistent relationships' between pesticide levels and harm to the insects could be found [FERA: URL: http://www.fera.defra.gov.uk/scienceResearch/scienceCapabilities/chemicalsEnvironment/documents/reportPS2371V4a.pdf].

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However, it turned out that the control colonies themselves were contaminated with the pesticides tested [147]. Further, thiamethoxam was detected in two out of the three bee groups tested, even though it was not used in the experiment. The major studies that have measured neonicotinoid residues in pollen collected by honeybees clearly show that neonicotinoids are found in pollen all over the year and in all studied regions, not only after the sowing or during the flowering period [89,91,96]. With the present scale of use, it will be very difficult to find a control site where bees cannot come into contact with neonicotinoids.

Given all the major limitations to the reliability of outcomes of field studies, it is recommendable to give more weight in the risk assessment to reproducible results from controlled lab studies and use the ratio between the environmental concentration and the no effect concentration as the main risk indicator [40,42]. It could perhaps be linked to modelling to explore how, and to what the degree, the various well-known sublethal effects on individual bees can weaken the colony [148].

A key aspect in honeybee biology is that the colony behaves as a 'superorganism' [149]. In a colony, sufficient membership, so that the number of organisms involved in the various tasks to maintain that colony, is critical, not the individual quality of a task performed by an individual bee. Varying between winter and summer, the 10,000–60,000 honeybees that typically form a colony function as a cooperative unit, maintaining intraorganismic homeostasis as well as food storage, nest hygienic, defence of the hive, rearing of brood etc. Hence, sublethal effects affecting the number of individuals that perform specific functions, can influence the functioning of the whole colony. In a simplified theoretical modelling approach, colony failure can be understood in terms of observed principles of honeybee population dynamics [150]. A colony simulation model predicts a critical threshold forager death rate above which rapid population decline is predicted and colony failure is inevitable. High forager death rates draw hive bees towards the foraging population at much younger ages than normal, which acts to accelerate colony failure [150].

Synergistic effects: pesticide–pesticide and pesticide–infectious agents

A synergy occurs when the effect of a combination of stressors is higher than the sum of the effect of each stressor alone. When neonicotinoids are combined with certain fungicides (azoles, such as prochloraz, or anilides, such as metalaxyl) or other agrochemicals that block cytochrome P450 detoxification enzymes, their toxicity increases by factor from 1.52 to 1141 depending on the combination [151,152]. The strongest synergism has been found for triflumizole making thiacloprid 1141 times more acutely toxic to honeybees [151]. This synergistic effect is the subject of patents by agrochemical companies [152,153].

Synergy has also been demonstrated for neonicotinoids and infectious agents. Prolonged exposure to a non-lethal dose of neonicotinoids renders beehives more susceptible to parasites such as *Nosema ceranae* infections [39••,154••,155•,156]. This can be explained either by an alteration of the immune system or by an impairment of grooming and allogrooming that leads to reduced hygiene at the individual level and in the nest, which gives the pathogens more chances to infect the bees. The same mechanism, where the balance between an insect and its natural enemies is disturbed by sublethal exposures to neonicotinoids that impairs grooming, is well known and often used in pest management of target insects [157–161].

Conclusion and prospects

In less than 20 years, neonicotinoids have become the most widely used class of insecticides. Being used in more than 120 countries in more than 1000 different crops and applications, they now account for at least one quarter of the world insecticide market. For pollinators, this has transformed the agrochemical landscape to one in which most flowering crops and an unknown proportion of wild flowers contain varying concentrations of neonicotinoids in their pollen and nectar. Most neonicotinoids are highly persistent in soil, water and sediments and they accumulate in soil after repeated uses. Severe surface water pollution with neonicotinoids is common. Their systemic mode of action inside plants means phloemic and xylemic transport that results in translocation to pollen and nectar. Their wide application, persistence in soil and water and potential for uptake by succeeding crops and wild plants make neonicotinoids bioavailable to pollinators in sublethal concentrations for most of the year. This results in the frequent presence of neonicotinoids in honeybee hives. Neonicotinoids are highly neurotoxic to honeybees and wild pollinators. Their capacity to cross the ion-impermeable barrier surrounding the central nervous system (BBB, blood–brain barrier) [7•] and their strong binding to nAChR in the bee's central nervous system are responsible for a unique chronic and sublethal toxicity profile. Neonicotinoid toxicity is reinforced by exposure time. Some studies indicate a non-monotonic [162•] dose–response curve at doses far below the LD50. Mass bee dying events in spring from acute intoxication have occurred in Germany, Italy, Slovenia and France during pneumatic sowing of corn seeds coated with neonicotinoids. Bees that forage near corn fields during sowing get exposed to acute lethal doses when crossing the toxic dust cloud created by the sowing machine.

At field realistic exposure levels, neonicotinoids produce a wide range of adverse sublethal effects in honeybee colonies and bumblebee colonies, affecting colony performance through impairment of foraging success, brood

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and larval development, memory and learning, damage to the central nervous system, susceptibility to diseases, hive hygiene etc. Neonicotinoids synergistically reinforce infectious agents such as *N. ceranae* and exhibit synergistic toxicity with other agrochemicals. The large impact of short term field realistic exposure of bumblebee colonies on long term bumblebee queen production (85% reduction) could be a key factor contributing to the global trends of bumblebee decline. Only a few studies assessed the toxicity to other wild pollinators, but the available data suggest that they are likely to exhibit similar toxicity to all wild insect pollinators. The worldwide production of neonicotinoids is still increasing. In view of the vital importance of the service insect pollinators provide to both natural ecosystems and farming, they require a high level of protection. Therefore a transition to pollinator-friendly alternatives to neonicotinoids is urgently needed for the sake of the sustainability of pollinator ecosystem services. The recent decision by the European Commission to temporary ban the use of imidacloprid, thiamethoxam and clothianidin in crops attractive to bees is a first step in that direction [163].

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Environmental relevant concentrations of imidacloprid negatively affects ovary development and fecundity in bumble bees. Dietary imidacloprid at 1 $\mu g\ l^{-1}$ is capable to reduce brood production by one third. The causal speculations include a feeding reduction linked to toxic exposure and/or alteration of the social communication among bumblebee workers.

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