果的发现不会对玉米产生经济方面的影响（Lumbierres *et al*.，2004）。

与以上报道的结果相反，Van Frankenhuyzen 和 Nystrom（1999）数据库列出了 Ahmad 等（1989）发表的论文，该论文报道，来自于苏云金芽孢杆菌变种 galleriae 的 Cry2Ab 对埃及伊蚊有毒，说明抗虫棉花 Bollgard II 中存在的 Cry2Ab 可能对双翅目昆虫有毒。相反，Widner 和 Whiteley（1989，1990）的研究表明，Cry2Ab 对双翅目昆虫无毒。关于 Cry2Ab 对双翅目昆虫毒性所出现的两种明显不同的结果，可能与测试步骤有关。Dankocsik 等（1990）将毒素溶于水中，并将蚊子幼虫浸入到溶液中；而 Ahmad 等（1989）将幼虫浸入到水中，其中水含有表达这一毒素的巨大芽孢杆菌（*Bacillus megaterium*）。目前，还不清楚这些测试方法是否合适，但必要时应当用饲喂方式来检测 Cry2Ab 对双翅目昆虫的毒性，如果双翅目昆虫有可能接触表达该毒素的植物材料，还应评估其对双翅目昆虫的风险。

取食了 Bt 棉花（Cry1A）后，第 1 代和第 2 代棉蚜的生殖历期变短、最长寿命缩短、存活率和最大生殖率降低（Liu et al.，2005a）。不过，第 2 代或第 3 代蚜虫种群很快就会克服这些不良影响，第 1 代取食 Bt 棉花的蚜虫生殖历期较长，而第 3 代的存活率和最大生殖力较高。然而，这 3 个形态参数的不对称波动，说明 Bt 棉花对蚜虫的胁迫性很高（Liu *et al*.，2005a）。这些研究表明，评价 Bt 棉花对非靶标植食性昆虫的影响时，重要的是应用作物特异性来测试致死和亚致死参数，对重点研究的生物进行几代和几个发育历期的研究，并测试该 Bt 全植株接触对生物的影响。

用各种 δ-内毒素对几种代表性的有益昆虫进行分析，已经提交的对非靶标昆虫研究的结果，为 Bt 植物的登记提供了技术支持。选用这些昆虫是因为在某些情况下，这些昆虫常作为捕食性或寄生性动物用于害虫的综合治理或生物防治。另外，在对农药进行评价时，这些昆虫具有长期安全使用的历史。此外，这些物种已经适应实验室的环境，可用有效的标准方法进行检测。

蜜蜂（意大利蜜蜂 *Apis melifera*）可能是测定传统农药影响时研究最多的非靶标昆虫，因此野生生物测试实验室在用蜜蜂进行室内测试方面具有丰富的经验。不过，由于 δ-内毒素主要影响靶标昆虫的幼虫，因此现在测试重点放在了毒素对蜜蜂幼虫的影响上，这就要求建立一些新方法。在为了支持 Bt 植物登记而提交的新的研究结果中，未观察到对蜜蜂产生不良影响。Cry1Ab 和 Cry1Ac 对蜜蜂（用毒素或含毒素的花粉处理蜜蜂幼虫和成虫）没有不良影响（Monsanto 和 Novartis，1996b；Mycogen 和 Novartis，1995c；Monsanto，1995b）。玉米花粉中的 Cry9C 对蜜蜂成虫或瓢虫也没有不良影响（Plant Genetic Systems，1998a、1998c）。在有关蜜蜂幼虫的两篇研究中，Cry3A 对蜜蜂幼虫没有影响（Monsanto，1995a、1995e）。在蜜蜂幼虫发育至成虫过程中，让蜜蜂接触 Cry1F 毒素或含 Cry1F 毒素的花粉，结果表明 Cry1F 对蜜蜂没有影响（Mycogen 和 Pioneer，2001a、2001c、2001e）。类似的研究在蜜蜂幼虫至成虫的发育中进行，Cry2Ab2 和 Cry3Bb1 对蜜蜂没有毒性（Monsanto，2001c、2001d、2002a）。已经证明 Cry34Ab1/Cry35Ab1 蛋白对蜜蜂幼虫发育没有不良影响。对 3～5 日龄蜜蜂幼虫进行如下处理未见不良影响：①用 2mg 表达 Cry34Ab1/Cry35Ab1 蛋白的玉米花粉处理；②用 5.6μg Cry34Ab1 和 Cry35Ab1 蛋白混合物处理；③用 3.4μg Cry34Ab1 蛋白处理；④用 2.8μg

Cry35Ab1 蛋白处理（Mycogen and Pioneer，2005i）。

一系列试验说明 Cry1A 毒素对普通草蛉 *Chrysoperla carnea* 幼虫具有毒性，从而说明利用新技术建立试验方案也存在很大的困难。Hilbeck 等（1998b）采用两种不同的无选择饲喂策略，用纯化的 Cry1Ab 毒素对普通草蛉 *C. carnea* 进行了生测。它们在每 ml 饲料中直接加入 100 μg 毒素，发现普通草蛉 *C. carnea* 死亡率为 57%，而对照饲料饲喂的普通草蛉 *C. carnea* 死亡率为 30%。而用另一饲喂策略进行的研究中，将不含毒素的鸡蛋作为第一食品来源，然后将幼虫放入加有或不加毒素的饲料介质中，处理和对照的死亡率分别为 29%和 17%。在另一试验中，Hilbeck 等（1998a）报道用取食 Bt 玉米的猎物饲喂 *C. carnea* 幼虫，发现普通草蛉 *C. carnea* 幼虫死亡率增加，发育历期也略有增加。猎物为鳞翅目靶标昆虫欧洲玉米螟 *Ostrinia nubilalis* 和鳞翅目非靶标昆虫棉贪夜蛾 *Spodoptera littoralis* 幼虫。将这两种猎物的数值进行平均（猎物间的差异不显著），发现用取食 Bt 玉米的猎物饲喂普通草蛉 *C. carnea* 幼虫，其死亡率为 62%；而用取食非 Bt 玉米的猎物饲喂的幼虫，其死亡率为 37%。在接下来的研究中，Hilbeck 等（1999）在猎物的食物中加入多种浓度的 Bt 玉米，利用生测系统比较了 Cry1Ab 毒素、原毒素和 Cry2A 毒素的影响，从而详细分析了猎物介导的 Cry1Ab 毒素对普通草蛉 *C. carnea* 的影响。据他们报道用最高浓度的 Cry1Ab（100 μg/g 饲料）饲喂棉贪夜蛾 *S. littoralis*，然后用棉贪夜蛾 *S. littoralis* 饲喂普通草蛉 *C. carnea*，结果普通草蛉 *C. carnea* 的死亡率为 78%，而对照的死亡率仅为 26%。

美国国家研究理事会（National Research Council，NRC，2000）对 Hilbeck 等（1998a；1998b）的研究进行了综述，认为其报道的影响是饲养策略及其所提供毒素量的不同所致。他们认为应当进行田间试验来评价 Bt 作物对天然捕食动物的影响，并引用了如下事例，即在 1 个 2 年期的小规模田间试验中，Bt 玉米与非 Bt 玉米的田间天敌没有差异（Pilcher *et al.*，1997）。后来进行了大规模试验（见本节关于对非靶标生物的风险）。在 Hilbeck 等（1998b；1999）的工作中，无选择饲料试验采用了高剂量毒素对两种绿草蛉（Hilbeck *et al.*，1998b）和捕食动物（Hilbeck *et al.*，1999）进行了测试。不过，这些试验不可能区分哪些影响是由于摄入毒素引起的，哪些是由于寄主品质降低引起的。由于需要考虑猎物的忌避行为和替代猎物等行为机制，因此田间试验结果很难与 Hilbeck 的研究结果吻合。最近的一项研究也没有发现高剂量的 Cry1Ab 对绿草蛉幼虫产生直接的毒性（Romeis *et al.*，2004）。Romeis 等（2004）指出，Cry1Ab 对普通草蛉 *C. carnea* 的影响可能是由饲料的品质引起，而不是直接产生毒性。不过，试验设计的差异使得直接比较这两个研究结果有待进一步解释。

尽管在以上的研究中观察到了差异，但在建立实验室测试手册时，有必要利用更具代表性的接触技术，这种代表性接触技术可以更好地反映接触情况，包括通常在实验室条件下无法饲养的非靶标昆虫。例如，Dutton 等（2003）关于捕食性节肢动物风险评估的综述认为，应当对这些捕食动物进行评估，评估的方法是根据节肢动物的取食习性进行实验室测试和接触评估，必要时进行田间试验。Andow 和 Hilbeck（2004）建议采用综合生态全植株评估策略。美国环境保护局的科学顾问团（2002 年 8 月 7 日）认为：由于普通草蛉 *Chrysoperla carnea* 难以充分接触毒素，且用普通草蛉进行实验室测试难度大，所以普

通草蛉的食物测试很复杂。因此，美国环境保护局现在正采用其顾问团的建议，将小花蝽 *Orius insidiosus* 选作合适的测试对象。在美国，玉米田中的小花蝽属昆虫通常取食卵，也是典型的花粉取食者。

Cry1Ab，Cry1Ac 和 Cry3A 对瓢虫成虫、绿草蛉幼虫（直接接触）和寄生蜂没有影响（Monsanto，1995a、1995b；Monsanto 和 Novartis，1996a）。玉米花粉中的 Cry9C 对瓢虫没有影响（Plant Genetic Systems，1998c）。将 Cry1F 以毒素形式饲喂给绿草蛉幼虫、寄生蜂和瓢虫成虫，结果表明没有产生影响（Mycogen 和 Pioneer，2001c、2001e）。用 Cry1F 饲喂帝王蝶幼虫时，未见幼虫死亡，但在高剂量下（30，000 ng/ml 饲料）幼虫的生长受到一些抑制（Hellmich *et al.*，2001）。对 Cry2Ab2 和 Cry3Bb1 的研究表明：它们对瓢虫成虫和绿草蛉幼虫没有影响（Monsanto，2001c、2002a）。此外，在用 Cry3Bb1 花粉饲喂瓢虫（Monsanto，2002d）以及用 Cry3Bb1 花粉对另外两种不同的瓢虫进行的饲喂研究（Monsanto，2002b；Duan *et al.*，2002）中，发现毒素对从幼虫至成虫阶段的瓢虫没有影响。类似地，Cry34Ab1/Cry35Ab1 蛋白对绿草蛉幼虫没有毒性（Mycogen 和 Pioneer，2005b）。用 50%的玉米螟卵和表达 Cry34Ab1/Cry35Ab1 蛋白的 50%的玉米花粉饲养瓢虫幼虫，结果未见影响（Mycogen 和 Pioneer，2005a）。

短期实验室研究表明，4 种鳞翅目昆虫对 Cry1Ac 敏感，但是用高于转基因棉花花粉和花蜜田间浓度 100 倍的纯化 Cry1Ac 来饲养 6 种非靶标昆虫和 4 种有益昆虫（Sims，1995），结果没有产生毒性。在实验室研究中，测试的大多数有益天敌没有因为取食 Cry1A 毒素或转 *Cry1A* 基因的植物材料而受到不良影响，这些天敌如 Orius spp.，*Geocoris* spp.，*Cyrtorhinus* spp.，*Nabis* spp. 和 *Zelus* spp.，*Coleomegilla* spp.，*Propylea* sp.（如 Pilcher *et al.*，1997；Zwahlen *et al.*，2000；Al-Deeb *et al.*，2001；Bernal *et al.*，2002a；Bai *et al.*，2005）。同样，也未检测到 Cry3A 和 Cry3B 对小花蝽属 *Orius* sp. 和草盲蝽属 *Lygus* spp. 和 *Coleomegilla* sp. 产生不良影响（Riddick 和 Barbosa，1998；Armer *et al.*，2000；Duan *et al.*，2002；Lundgren 和 Wiedenmann，2002；Kalushkov 和 Nedved，2005）。

Romeis 等（2006b）报道用 Bt 植物材料直接饲喂天敌，结果未见不良影响，但是却发生了第三级营养阶层效应，即捕食动物或寄生蜂如果取食了用 Bt 饲养的猎物，则捕食动物或寄生蜂可能会受到不良影响。Romeis 等（2004；2006b）认为这种三级营养阶层效应是由猎物的营养质量差异所致。Ponsard 等（2002）检测了 Bt 棉花和摄入了 Bt 棉花的鳞翅目猎物对棉花害虫的 4 种重要鳞翅目捕食动物存活所产生的影响。暗小花蝽 *Orius tristicolor* 和蝉大眼长蝽 *Geocoris punctipes* 的寿命显著降低（降低了 27%～28%），*Nabis* sp. 和 *Zelus renardii* 没有影响（Ponsard *et al.*，2002）。取食转 Bt 基因的水稻花粉后，龟纹瓢虫 *Propylea japonica* 的存活率降低（Bai *et al.*，2005）。

如果寄主用 Bt 植物或 Bt 饲料饲喂，则膜翅目寄生蜂通常会受到不良影响，这大部分是由寄主品质降低所导致（Lövei 和 Arpaia，2005；Romeis *et al.*，2006b）。在实研室内给膜翅目寄生蜂喂食用 Cry1Ac 棉花饲养的大豆夜蛾 *Pseudoplusia includens*，结果发现该寄生蜂的发育延迟，其原因可能是毒素对寄主产生了亚致死效应（Baur 和 Boethel，2003）。同样地，中国棉铃虫上重要的寄生蜂斜纹夜蛾侧沟茧蜂 *Microplitis mediator* 寄生

了用 Bt 棉叶粉（Cry1Ac）饲养的棉铃虫 *Helicoverpa armigera* 后，其存活率降低且生长受到抑制。如用 Gy1Ab Bt 玉米饲喂棉贪夜蛾 *Spodoptera littoralis* 的幼虫，则螟蛉绒茧蜂 *Cotesia marginiventris* 的存活率、发育历期和茧的重量受到显著的不良影响（Vojtech *et al.*，2005）。Prütz 及同事研究了用 Bt 棉叶（Cry1Ab）饲养的寄主斑禾草螟 *Chilo partellus* 对寄生蜂螟黄足盘绒茧蜂 *Cotesia flavipes* 的影响，结果发现寄生蜂体重下降、很难完成发育（Prütz 和 Dettner，2004；Prütz *et al.*，2004）。毒素对寄生蜂螟黄足盘绒茧蜂 *C. flavipes* 产生的不利影响，进而会对四级营养水平产生次级影响。在用 Bt 饲养的斑禾草螟 *C. partellus* 上发育的螟黄足盘绒茧蜂 *C. flavipes* 被雌性重寄生蜂黑棒啮小蜂 *Tetrastichus howardi* 寄生后，重寄生蜂的体重降低，后代数量减少（Prütz *et al.*，2004）。寄生蜂菜蛾绒茧蜂 *Aphidius nigripes* 在用 Bt 马铃薯（Cry3A）饲养的非靶标蚜虫上发育时，其存活率降低、成蜂身体变小（Ashouri *et al.*，2001）。用 Bt 玉米（Cry9c）饲喂的寄主，寄生蜂 *Parallorhogas pyralophagus* 的发育历期、寿命和死亡率都受到了不良影响，但是性比、抱卵量、成蜂身体的大小不受影响（（Bernal *et al.*，2002b）。总之，对植食性靶标昆虫和植食性非靶标昆虫的亚致死效应可对寄生蜂产生影响，并且这种影响可能通过改变寄生蜂一寄主的种群动力学，而影响寄生蜂所提供的生物防治程度，受到次级影响的昆虫可能也包括次要害虫以及在下茬作物或相邻作物中发生的害虫（Bernal et al.，2002b）。不过，还应针对有益昆虫在害虫控制中所起的作用以及相关昆虫的种群动态来评估亚致死效应（Mendelsohn *et al.*，2003；Romeis *et al.*，2006b）。

人们已经检测了 Bt（Cry3Aa）马铃薯对瓢虫和蚜虫的潜在影响。Dogan 等（1996）的一项研究中在蚜虫取食了含苏云金芽孢杆菌变种 *tenebrionsis* δ-内毒素（Cry3 毒素）的马铃薯叶片后，再将其饲喂给锚斑长足瓢虫 *Hippodamia convergens* 幼虫和成虫。瓢虫属于鞘翅目，因此是 Cry3 毒素的潜在靶标，这一研究旨在测定这些有益的捕食性昆虫是否会因蚜虫取食了 Bt 马铃薯而受到影响。结果表明并未发现通过捕食蚜虫而产生对瓢虫的影响。由于研究中没有观察到不良影响，所以其机制并不明确。据了解，蚜虫在 Bt 作物上取食汁液时几乎不摄取 Bt 毒素（Head *et al.*，2001；Raps *et al.*，2001；Dutton *et al.*，2002）。因此，不可能通过捕食蚜虫产生影响。Riddick 和 Barbosa（1998）发现，猎物 *Leptinotarsa decemlineata* 在取食了 Bt 马铃薯（Cry3A）后对捕食性瓢虫猫斑长足瓢虫 *Coleomegilla maculata* 没有产生不良影响。

用含 Cry1Ab 和 Cry1Ac 的棉花、含 Cry3A 的马铃薯对两种土壤节肢动物，即跳虫 *Folsomia candida* Willem 和甲螨 *Oppia nitens* Koch 进行测试，结果未见不良影响（L. Yu *et al.*，1997）。已提交的弹尾目昆虫研究结果，为申请 Cry1Ab、Cry1Ac、Cry1F、Cry3A、Cry9C、Cry2Ab2、Cry3Bb1、Cry34Ab1/Cry35Ab1 等转基因品种的商业登记提供了证据（见参考文献）。用植物以及微生物产生的 Cry1Ac 和 Cry9C δ-内毒素分别进行试验，结果未见不良影响（DEKALB，1997；Novartis 和 Monsanto，1996；Plant Genetic Systems，1998c）。在申请含 Cry1Ab 的转基因玉米产品登记而提交的材料中，有两种明显对立的研究结果：一个试验用 200 ppm 来源于重组大肠杆菌的 Cry1Ab 毒素进行测试，结果未观察到两种弹尾目昆虫（跳虫 *Folsomia candida* 和奇虫兆 *Xenylla* grisea）受到不良影响（Novartis 和 Monsanto，1996）；另一个研究用冻干的叶片提取物进行测试，结果发现每 kg 土中含 125 mg Cry1Ab 玉米叶片蛋白时可致跳虫 *Folsomia candida* 死亡。目前仍无法确定本研究中观察到的毒性是由叶片提取物中的 Cry1Ab δ-内毒素所致，还是由于其他蛋白的相互作用所致。利用 112 段所讨论的危害性方面数据，可进行最坏情况下的评估。用 200 ppm 的微生物 Cry3A 毒素对跳虫 *Folsomia candida* 和奇虫兆 *Xenylla grisea* 不产生影响（Novartis 和 Monsanto，1996）。在用 Cry1F 进行的 28 天慢性毒性试验和用 Cry2Ab2 和 Cry3Bb1 进行的饲喂试验中，未见不良影响（Mycogen 和 Pioneer，2001e；Monsanto，2001c、2002a）。由于 Bt 玉米的营养品质更高，因此球鼠妇 *Porcellio scaber* 用 Cry1Ab 玉米饲养后，其表现要好于用非转基因玉米饲养的昆虫（Escher *et al*.，2000）。一项实验室研究表明，用含 Cry3Bb1 和 Cry1Ab 的玉米花粉饲养 16 种步甲后，没有发现 Bt 毒素所产生的影响（Mullin *et al*.，2005）。用 930 μg Cry3Bb1 蛋白/g 饲料进行最大危害性剂量实验，结果这种饲料对步甲 *Poecilus chalcites* 的存活、发育和生长没有不良影响（Duan *et al*.，2006）。用取食了 Bt 玉米的猎物饲喂 *Poecilus cupreus* 幼虫，结果表明幼虫的死亡率高于用非 Bt 猎物饲养的昆虫。这些影响可能是由猎物介导的，但由于步甲幼虫可摄入 Bt 毒素，因此也不能排除直接影响的可能性（Meissle *et al*.，2005）。用 Cry3Bb1 和 Cry1Ab 玉米对 16 种步甲进行的一项研究中，没有发现 Bt 毒素（接触花粉）所产生的影响，而用新烟碱类杀虫剂处理的种子饲养该甲虫，发现昆虫几乎全部死亡（Mullin *et al*.，2005）。

（2）非靶标生物对毒素的接触

在急性危害性测试中，如果发现某种生物受到了不良影响，接触分析则可以使它们进行风险评估。接触的途径有几种，可以接触转基因作物中的毒素，如果出现异型杂交的话，还可以接触野生近缘种中产生的毒素。不过，异型杂交的潜力具有作物特异性和地区特异性，各种作物已在相关的文章中做了很好的阐述。非靶标生物的接触取决于其生活环境、取食生态学和生命周期。可通过摄取 Bt 植物材料以及与土壤结合的 δ-内毒素直接接触，也可以通过食物链间接接触。从植物各部分 δ-内毒素的可能最大含量可估计可能的最大直接接触量。从提交美国环境保护局申请登记的数据来看，不同转基因植物、不同组织、不同龄期植株中毒素的浓度存在很大变化。几个转基因玉米商业品种中报道了 Cry1Ab δ-内毒素的表达量。其中一个转基因品种中最大表达量为 10.34 μg/g 叶片，4.65 μg/g 全植株，<0.09 μg/g 花粉（干重）（Monsanto，1995c；1995d）。另一个转基因玉米品种中，最大表达量为 4.4 μg/g 叶片，0.6 μg/g 全植株，7.1 μg/g 花粉（Mycogen and Novartis，1995d）。一个转基因玉米品种中 Cry3Bb1 的最大表达量为 450 μg/g 叶片，390μg/g 根，42 μg/g 花粉（干重）。

毒素的表达量最高的作物是表达 Gry9c 的玉米（Plant Genetic Systems，1998c）。在营养生长期植株各部分中的最高含量（以干重计）为：250.0 μg/g 全植株，175.0 μg/g 穗，44.0 μg/g 叶片，25.87 μg/g 根，18.6 μg/g 玉米粒，2.8μg/g 玉米秆，0.24μg/g 花粉。随着植株龄期的增加，δ-内毒素的含量降低，而且不会产生新的蛋白来取代已降解的蛋白。对全植株中 δ-内毒素（以干重计）的分析表明：营养生长期为 250 μg/g，花粉散落期为 230 μg/g，青贮期为 96μg/g，收获期为 22 μg/g。对于取食这些植物的生物，可直

接利用这些接触数据。但取食花粉的昆虫例外，这些生物应被视作靶标害虫。还有一些同时取食其他昆虫和植物的生物（如蝽科会接触到毒素捕食性动物），既可视为有益昆虫又可被视为潜在的植物害虫。此外，取食腐烂 Bt 植物材料的屑食者，以及取食植食性昆虫和取食 Bt 植物屑食者的捕食性动物也可接触到 δ-内素。

花粉是非靶标生物接触毒素的潜在来源。如在影响这一段所述，取食沉淀在植物上的花粉既可影响敏感性非靶标生物，也可影响害虫（Felke 和 Langenbruch，2001、2003；Felke *et al*.，2002）。在欧洲，大部分花粉在 7 月份散落（Zscheischler *et al*.，1990；Lang *et al*.，2004）。通常玉米的开花期持续 5～8 天，不过在有利条件下，大部分花粉在 2 天内可散落（Treu 和 Emberlin，2000；Wolt *et al*.，2003），但是在玉米田中花粉的散落期可持续 10～14 天（Treu 和 Emberlin，2000，Oberhauser *et al*.，2001）。玉米上可散落大量的花粉，Emberlin 等（1999）估计，一个玉米田块大致可产生 70 kg/英亩的玉米花粉。玉米花粉可通过风传播至 800 m（Treu 和 Emberlin，2000）甚至几千米的地方（Brunet *et al*.，2003），但是由于花粉的体积和重量大，因此只有不到 1%的花粉粒可落在离“花粉源”60 m 开外的地方（Raynor *et al*.，1972）。通常，大部分花粉在距玉米田边 10 m 的地方落下来，随着距离的增加，落下的花粉呈指数降低（Hansen *et al*.，2000；Wraight *et al*.，2000；Stanley-Horn *et al*.，2001；Zangerl *et al*.，2001；Lang *et al*.，2004；Li *et al*.，2005；Shirai 和 Takahashi，2005）。平均来说，1/3 的花粉飘到田梗边，落到蝴蝶的寄主植物上（Pleasants *et al*.，2001；Lang *et al*.，2004）。如果花粉含有对鳞翅目害虫具活性的 Bt 蛋白，且花粉密度超过了毒性阈值，则在一定范围内花粉会对生活在寄主植物上的蝴蝶幼虫产生不良影响（Felke 和 Langenbruch，2003；Zangerl *et al*.，2001；Dively *et al*.，2004）。要评价 Bt 玉米对田埂边蝴蝶幼虫的影响，需了解粮食作物上天然存在的玉米花粉密度、Bt 玉米花粉中毒素的浓度及其对蝴蝶幼虫的毒性（Lang *et al*.，2004）。

如果猎物（或花蜜）含有 δ-内毒素，则用取食 Bt 植物的猎物饲喂捕食动物可能会使捕食动物接触到 Bt 毒素。不同猎物所摄入的毒素量不同。例如，蚜虫吸食 Bt 植物（如 Cry1Ab 玉米）的汁液时，几乎不摄入 Cry 蛋白，其原因可能是玉米韧皮部汁液中不含 Bt 蛋白（Head *et al*.，2001；Raps *et al*.，2001；Dutton *et al*.，2002）。相反，取食 Bt 玉米的鳞翅目害虫，所摄入的 Cry1Ab 蛋白的量因不同昆虫而异（Head *et al*.，2001；Raps *et al*.，2001；Dutton *et al*.，2002；Vojtech *et al*.，2005）。而且，取食 Bt 植物的其他植食性昆虫可能会含有 δ-内毒素（Cry1Ab，Cry1Ac），如一种螨类、一种蓟马、一种膜翅目害虫和 1 种蛞蝓（Dutton *et al*.，2002；Howald *et al*.，2003；Obrist *et al*.，2005；Harwood 和 Obrycki，2006）。在取食腐烂 Bt 玉米的腐食性昆虫 *Porcellio scaber* 中，可检测到 Cry1Ab 毒素（Wandeler *et al*.，2002）。一项实验室研究表明，步甲取食被 Bt 污染的猎物后，死亡率高于对照（Meissle *et al*.，2005）。田间试验数据表明,非靶标植食性昆虫在 Bt 玉米田中可摄入 Bt 毒素。Harwood 等(2005）研究表明，蜘蛛目，瓢虫科和姬蝽科平均含 0.42～2.53 μg Bt 毒素/g 鲜重，Zwahlen 和 Andow（2005）测定的 Bt 毒素的含量为 6.4～117.3 μg/g 鲜重,在一些步行虫科昆虫 Bt 毒素的含量为 6.4～117.3 μg/g。

由于 Bt 植物在整个生长季节均表达内毒素，因此除非在整个生长季节一直使用 Bt 喷

雾剂，否则捕食动物通过取食 Bt 植物的猎物而接触毒素的量可能比 Bt 喷雾剂多。目前尚不清楚这些非靶标生物接触毒素后的表现；不过以上数据表明，非靶标生物可在田间长期接触 Bt 毒素。捕食者的表现尤其是对猎物的选择，可影响它们对 Bt 毒素的接触。在猎物选择试验中，*P. cupreus* 成虫不躲避含有 Bt 的猎物，甚至喜欢选择用 Bt 饲喂的棉贪夜蛾 *Spodoptera littoralis*（Meissle *et al.*，2005）。另一种步甲如大壶步甲 *Lebia grandis* 取食用 Bt 马铃薯叶片（Cry3A）饲喂的猎物数量，与取食用非 Bt 马铃薯饲喂的猎物数量一样多（Riddick 和 Barbosa，2000）。普通草蛉 *Chrysoperla carnea* 幼虫更偏好在非转基因玉米上取食的猎物，而不偏好在 Bt 玉米（Cry1Ab）上取食的猎物，这可能会降低这一捕食者对毒素的接触（Meier 和 Hilbeck，2001）。Rovenska 等（2005）在实验室研究表明，植食性螨二斑叶螨 *Tetranychus urticae* 偏好取食表达 Cry3Bb 毒素的茄子。同时，捕食性昆虫智利小植绥螨 *Phytoseiulus persimilis* 取食用 Bt 茄子饲养的螨类数量少。

作物收获之后，Bt 植物残留物仍然留在田间。生长季节过后，Cry1Ab 仍可在 Bt 玉米叶片和 Bt 玉米田的土壤中检测到，只是多数情况下浓度很低（Hopkins 和 Gregorich，2005；Zwahlen *et al.*，2003b；Baumgarte 和 Tebbe，2005）。通过收获时全植株中 δ-内毒素的表达量，可以预测土壤生物的最高接触量。就毒素表达量很高的 Cry9C 转基因植株而言（Plant Genetic Systems，1998c），收获时 δ-内毒素的含量为 99 g/英亩（假定 1 英亩种植了25 000株玉米），预计环境中的浓度（EEC）为在 15 cm 深层土中含 0.11 mg/kg。为了申请产品的商业化而利用敏感昆虫烟蚜夜蛾 *Heliothis virescens* 进行的实验室生测表明，植物产生的 Cry9C δ-内毒素在测试土壤中的生物降解期超过 42 天，半衰期为 4.5 天（Plant Genetic Systems，1998b）。这些结果与微环境测试的 Cry1Ac 在棉花中的半衰期为 2～46 天相吻合（Palm *et al.*，1996）。类似地，就上面所介绍的第 2 个 Cry1Ab 转基因植株而言，如果将收获后产生的衰老玉米植株耕入到土壤 6 英寸处，则土壤中的最大浓度为 4.2×10^{-4} mg Cry1Ab/kg 土 [190 mg Cry1Ab/英亩×1/0.5 提取效率×1 英亩（6" 深）/9.08×10^{5} kg 土＝ 4.185×10^{-4} mg Cry1Ab/kg 土]。不过，土壤中来自苏云金芽孢杆菌的 δ-内毒素可与腐殖酸、泥土以及可保护毒素降解的土壤有机矿物质结合。不过，由于植物材料的腐烂，Bt 毒素在土壤可能分布不均（Baumgarte 和 Tebbe，2005；Hopkins 和 Gregorich，2005）。

Vettori 等（2003）在意大利撒丁岛橡胶林中喷施 Bt 商品喷雾剂（FORAY 48B®）防治舞毒蛾，并研究了 Bt 在土壤中的持效期和活性。结果表明，苏云金芽孢杆菌 *kurstaki* 亚种及其毒素可在土壤中保持较长时间的持效性（*Btk* 的持效期至少为 88 个月，其毒素的持效期至少为 28 个月）。一项对 6 个非转基因玉米品系以及 2 个 Cry1Ab 品系进行的实验室研究表明，生物分解者不受 Bt 毒素的影响，尽管其中有 1 个 Bt 品系的降解速度不如其他品系，但非转基因品系间也存在相似的差异。一篇论文（Zwahlen *et al.*，2003a）报道了玉米干草中的 Cry1Ab 在田间的降解速度比在实验室中慢，另一篇文章（Zwahlen *et al.*，2003b）测定了装袋埋入土壤 200～240 天的玉米叶片中 Cry1Ab 含量，以及 200～240 天后土表面植物材料中 Cry1Ab 含量，结果表明只要植物没有降解，Cry 蛋白就可保留于土壤中。最近，室内试验分析了表达 Bt 毒素不同植物的降解问题，讨论了毒素的降解与木质素含量的关系，以及对环境所产生的影响。Bt 植物的分解速度通常比非 Bt 植物

慢。不过，这一影响显然与木质化无关，也与土壤中微生物活性的降低无关（Flores *et al*.，2005）。

最近的研究表明，Bt 玉米中的 Cry1Ab 毒素会释放到土壤根分泌物和液体生长环境中（Saxena *et al*.，1999；Saxena and Stotzky，2000）。在第 1 个研究中，Saxena 等（1999）表明在液体培养基上培养 7 天后，转基因玉米中的 Cry1Ab 以截短活性形式释放出来，但 25 天后却检测不到，其原因可能是微生物或植物介导了 Cry1Ab 的降解。这一研究以及随后的研究（Saxena 和 Stotzky，2000）表明，毒素可从不同类型土壤种植的转基因玉米根中释放出来。在这 2 个试验中，Stotzky 及其同事利用酶联免疫分析（ELISA）和烟草天蛾幼虫生测对毒素进行了测定，并在第 1 个试验中用 SDS-PAGE 对毒素进行了测定。他们认为，由于这些玉米植株表达截短形式的 Cry1Ab 毒素，因此不存在毒素特异性的溶解和蛋白质水解等过程。由于毒素在土壤中含量的田间调查数据很少，因此可能存在毒素对土壤微生物的非预期影响。土壤中来自于苏云金芽孢杆菌的 δ-内毒素以及植物所产生的 δ-内毒素可与腐殖酸、泥土和有机矿物质复合物结合，从而可保护 δ-内毒素免受土壤微生物的降解（Saxena 和 Stotzky，2000；Stotzky，2000；Crecchio 和 Stotzky，2001；Saxena *et al*.，2002a、2002b）。在几个月内，仍可在土壤中测定到这些毒素（Tapp 和 Stotzky，1995a、1997）；如果毒素与土壤粒子结合，则毒素可在实验室条件下保持活性（Tapp 和 Stotzky，1995b）。不过，另外一项室内生测结果表明，将植物所产生的 δ-内毒素加入到自然土壤中后，δ-内毒素活性的降低程度与没有被土壤微生物群落结合降解的速度相等（Palm *et al*.，1996；Pratt *et al*.，1993）。例如，用烟芽夜蛾 *Heliothis virescens* 生测并定量分析 Cry1F 蛋白在土壤中的快速降解，结果表明 Cry1F 毒素在土壤中的半衰期为 0.6 天（Herman *et al*.，2001；2002b）。类似地，利用南方玉米根虫 *Diabrotica undecimpunctata howardi* 分析了 Cry34Ab1/Cry35Ab1 在土壤中的降解，结果表明这一双毒素的半衰期短于 4 天（Herman *et al*.，2002a）。

如果通过细胞分泌装置从根中浸提出活性形式的毒素，则土壤中存在的毒素浓度比从渗漏细胞中释放的浓度高，或比通常的植物动力学浓度高。例如，生长期间从根尖细胞流出的毒素或一些根所降解的毒素。这是分析转基因作物风险时所需考虑的重要因素（USEPA，2000）。由于 Cry1Ab 毒素不含信号肽这一真核细胞分泌所需的 N 末端序列，因此植物细胞不会分泌这一毒素（Vitale 和 Denecke，1999）。“浸提物”更多地来源于生长期从根尖细胞流出的或一些根所降解的毒素。在一个多年的试验中没有发现种植 Bt 棉的土壤中存在 Cry1Ac 蛋白，因此这一影响可能是玉米特有的（Head *et al*.，2002）。多个田间试验表明土壤中未发现 Cry1Ac（Head *et al*.，2002），或 Bt 棉田和 Bt 玉米田土壤中未发现 Cry1Ac 蛋白（Dubelman *et al*.，2005），因此这一现象以及前面所提到的试验似乎无法预测土壤中 Bt 的含量。此外，在监测试验中，在美国 5 个玉米种植区连续 3 年或 3 年以上种植 MON810 或 Bt11 玉米，从田间收集土壤，利用有效的昆虫生测进行统计分析。结果发现 Cry1Ab 存在于授粉期所有试验点的土壤中，含量十分接近于检测限（LOD=0.03 μg/g）。收获后不久，短暂残留的毒素消失。在其它 4 个试验点以及在玉米生长季节中或之后的任一时间均未检测到 Cry1Ab 蛋白（Dubelman *et al*.，2005）。从堪萨斯州进行 MON863 玉米（商品名为 YieldGard 的抗根虫玉米）多年田间试验的小区中

收集土壤，用 Cry3Bb1 ELISA 试剂盒对土壤进行分析，结果只有 1 个样品中残留有微量的 Cry3Bb1 蛋白（<0.007 μg/g），这一残留蛋白很快消失。Cry3Bb1 蛋白在土壤中不持续存在，而且没有检测到对土壤表面以及次表面的节肢动物产生影响（Ahmad *et al.*，2005）。利用昆虫生测（LOD ＝0.008 μg/g），在多个转基因抗虫棉 Bollgard 田中对 Cry1Ac 蛋白进行多年监测试验，结果表明在耕地后 3 个月收集的土壤样品中未检测到 Cry1Ac 蛋白（Head *et al.*，2002）。

（3）对非靶标生物的风险

由于 Bt δ-内毒素具有选择性，与靶标生物具有近似分类地位的非靶标生物最可能受到影响。如果猎物取食了转基因植株，则捕食性昆虫可能会接触 δ-内毒素。不过，它们的猎物可能对 δ-内毒素敏感，因此这些猎物品质差或者不能用作捕食性昆虫的饲料。通常地，用任何一类杀虫剂进行植食性作物害虫防治时，即使杀虫剂不直接影响这些捕食性昆虫，由于猎物的数量减少，捕食性昆虫还是会受到不良影响。如果生物防治中昆虫的释放与 Bt 作物的使用同时进行，则可了解捕食性昆虫种类以及 Bt 作物对捕食性昆虫种群的影响，有助于害虫综合治理的规划。

田间调查可很好地指示毒素对非靶标昆虫的总体影响，但是田间调查通常很难设计和控制，并且田间调查和分析的成本较高。检测物种或类群丰度变化的能力很大程度上取决于试验设计和统计检验能力（Lang，2004）。美国环境保护局认为，如果我们将田间检测转基因玉米对非靶标节肢动物的影响作为一种更高级别的风险评估标准，这依赖于来自实验室的测试结论。美国环境保护局的一个科学顾问团（USEPA，2003）认为："选择适当的单一物种的Ⅰ级实验室测试如果表明毒素没有产生有害影响，则这一试验足以用来进行短期的危害性评估；如果这些测试表明毒素产生了毒性，则应当进行田间试验（更高等级的测试见 OPPTS 微生物测试指南中的描述），但需用具有适当统计检验能力的多年田间试验数据来确定长期的生态效应"。这就需要对合适的田间小区进行测试，避免节肢动物在更小的区间出入而引起取样误差（Prasifka *et al.*，2005）。

现在已经进行了很多田间试验，且大多数试验结果已经发表。对有益节肢动物（包括瓢虫、捕食性步甲、褐草蛉、绿草蛉、花蝽、猎蝽、姬蝽，寄生蜂、豆娘、蜻蜓和蜘蛛）进行的田间调查表明，除了规律不一致的一些微小变化之外，转 Cry1F 基因玉米田与非转基因玉米田中的昆虫数量没有显著差异（Mycogen and Pioneer，2001e）。对 Cry3Bb1 玉米进行了 2 年田间试验，共收集到 16 个目、46 个科156 572个生物，其中无脊椎动物包括害虫、捕食者、寄生蜂、腐质动物和分解生物。未检测到 Bt 玉米对非靶标无脊椎动物的影响（Monsanto，2002e、2002g）。2000 年开始在农场范围内进行试验，作为西班牙对 Bt 玉米（Bt176）特定监测计划的部分内容，评估 Bt 玉米对捕食性节肢动物的潜在影响。数据表明 Bt 玉米对天然存在的捕食动物没有不良影响（De La Poza *et al.*，2005），对某些玉米害虫包括蚜虫、蝗虫、地老虎、线虫和金针虫没有不良影响（Pons *et al.*，2005）。

如果玉米螟 *Ostrinia nubilalis* 是 Bt 玉米田中需要防治的靶标害虫，则其专性捕食动物和拟寄生物的种群多样性将降低（Bourget *et al.*，2002）。Siegfried 等（2001）发现 Bt 玉米田中玉米螟专性天敌的种群丰度低于非 Bt 玉米田。在法国进行的一个田间测试中，Bt 玉米对整个生长季节收集的非靶标植食性昆虫或有益节肢动物产生的影响可以忽略不

计（Candolfi *et al*.，2004）。不过，在田间试验中将杀虫剂和 Bt 玉米处理靶标害虫所受到的影响进行比较时发现，广谱性杀虫剂（如拟除虫菊酯）降低了大量捕食性昆虫和拟寄生昆虫的丰度，他对玉米螟的作用不特异（Dively 和 Rose，2003；Candolfi *et al*.，2004）。用 Cry1Ab 和 Vip3 玉米进行的 3 年田间测试中，在 Bt 玉米对群落的影响显著低于杀虫剂，但这些改变不会延续至下一生长季节（Dively，2005）。用 Cry1Ab 进行的 2 年田间试验表明，Bt 玉米田中姬猎蝽属捕食性昆虫略为减少，而其他非靶标植食性或捕食性节肢动物种群没有减少。玉米田中极为不常见的姬蝽科昆虫似乎对猎物数量的降低产生了反应（Daly 和 Buntin，2005）。

在爱荷华州用欧洲玉米螟对 Cry1Ab 玉米（抗玉米螟为害）进行 3 年大规模田间测试中，未发现毒素对 4 种常见的捕食性昆虫（2 种鞘翅目昆虫、1 种异翅亚目以及 1 种脉翅目昆虫）产生影响，但是腰带长体茧蜂 *Macrocentrus cingulum* 这种专门寄生欧洲玉米螟的膜翅目寄生蜂在 Bt 玉米中的数量显著减少。已经表明这种特异性与非 Bt 玉米小区中种群密度增加有关（Pilcher *et al*.，2005）。在伊利诺伊州进行的 3 年田间试验中，调查了 Cry3Bb1 玉米（抗玉米螟）叶片上生活的节肢动物，发现生活在叶片上的任何非靶标节肢动物的相对丰度都没有受到不良影响，这些非靶标节肢动物包括捕食动物与寄生蜂，其中共抓获了14 000头，并进行鉴定。在伊利诺伊州对 Cry3Bb1 玉米同时进行的 3 年试验表明，与非 Bt 玉米相比，所有非靶标生物、地下昆虫种群的丰度都没有受到不良影响，其中地下昆虫类群包括蜘蛛目（螨类）、步行虫科（步甲）、隐翅虫科（隐翅虫），和食腐质动物（分解生物），如铗尾虫科（双尾虫），姬薪虫科（水龟虫），蚁科（蚂蚁），唇足纲（蜈蚣）、寡毛纲（蚯蚓）（Bhatti *et al*.，2005a）。

Bt 棉田中完成的几个田间测试发现，Bt 棉对次要半翅目害虫、蚜虫和天敌没有显著影响（Wang 和 Xia，1997；Fitt 和 Wilson，2002；Liu *et al*.，2002b；Wu 和 Guo，2003；Torres 和 Ruberson，2005；Head *et al*.，2005）。在亚利桑那州对 Cry1Ac 棉花进行为期 6 年的大规模田间试验表明，22 类在叶片上生活的节肢动物天敌中，有 5 类的数量较非 Bt 棉减少了 19%（Naranjo，2005a）。不过在同时进行的另一个 5 年田间试验中，检测了 Bt 棉是否对 3 种主要害虫的天敌产生了影响，结果发现 Bt 棉没有对捕食者造成潜在影响，但杀虫剂的应用却对捕食动物形成了一定的影响，这说明在 6 年实验中所观察到的影响对生态的冲击很小（Naranjo，2005b）。Sisterson 等（2004）进行的田间试验中，Bt 棉和非 Bt 棉小区间节肢动物的丰度没有差异，但是 Bt 棉小区中节肢动物的丰度比混合种植 Bt 和非 Bt 植物的小区低。在澳大利亚进行的 3 年田间试验中，在施用了农药的棉田中，有益节肢动物群落的物种丰度比 Cry1Ac 棉田和未施用农药的棉田低。与 Bt 棉田相比，未喷施农药的常规棉田中双翅目昆虫姬蝽和小叶蝉的数量略高（Whitehouse *et al*.，2005）。在中国进行的 3 年田间试验中，由于猎物的数量降低，因此 Cry1Ac 棉田中的瓢虫数量降低，而蜘蛛的密度增加。Bt 棉田中螨类不受影响，对蚜虫的影响在不同的年份存在差异（Men *et al*.，2004）。在 Bt 棉中节肢动物的总密度以及次要害虫种群密度增加，但是次要天敌种群的多样性降低（Men *et al*.，2003）。尽管没有用杀虫剂来防治 Bt 棉田中的主要害虫（棉铃虫），但由于要用杀虫剂来防治刺吸式害虫，因此这 3 年期间 Bt 棉田中杀虫剂应用的次数不少于非 Bt 棉田（Men *et al*.，2004）。这与澳大利亚的情况

相反，在10年间杀虫剂的应用减少了75%～85%（APVMA，2003），在棉花生长排灌区的河流和溪水中，主要花粉指标显著降低（NSW Dept. Land & Water Conservation，2001）。在中国进行的另一田间试验中，两种次要害虫（半翅目：盲蝽科）的密度在Bt棉田与非Bt棉田中没有差异，不过，未喷施农药的Bt棉田中螨类的危害显著高于喷施了农药的非Bt棉田，说明这些螨类成为转基因棉田中的主要害虫，可能需要采用另外的防治措施加以防治（Wu *et al.*，2002）。据中国发表的文章报道，室内测定的对天敌营养的不利影响因不同的Bt棉品种而异（Guo *et al.*，2004）；与进行了害虫综合防治的非Bt棉田相比，Bt棉田中的天敌数量增加、植食性害虫数量减少（Liu *et al.*，2002a）；转基因Bt棉田中捕食性节肢动物的数量高于非Bt棉田（包括进行了害虫综合防治的或化学防治的非Bt棉田）（Wan *et al.*，2002）。对迄今为止发表的田间测试进行的综述认为，对商业Bt棉进行的大规模试验没有发现其对非靶标生物产生了非预期的影响，只是在对靶标害虫进行有效的防治过程中节肢动物群落发生了微小的变化（Romeis *et al.*，2006b）。

如前所述，在大部分田间试验中未观察到Bt植物对无脊椎天敌产生影响。不过，也有一些例外的例子，在Bt处理后，一些主要生物的丰度降低，也有一些丰度增加，下面是具体的实例。在Bt棉（Cry1Ac、Cry2Aa）田中跳蛛的丰度降低（Whitehouse *et al.*，2005），而Bt玉米（Cry3Bb）、Bt棉（Cry1Ac、Cry2Aa）田和Bt马铃薯（Cry3A）田中蜘蛛的数量更多（Riddick *et al.*，2000；Men *et al.*，2004；Bhatti *et al.*，2005b；Men *et al.*，2004）。经常发现Bt棉（Cry1Ac、Cry2Aa）、Bt玉米（Cry1Ab）田中食虫蝽受到影响，其数量减少（Daley 和 Buntin，2005；Naranjo，2005a；Whitehouse *et al.*，2005）。Bt玉米（Cry1Ab）田中食虫蝽的数量增加（Musser 和 Shelton，2003）；而其他实验结果不一（Wold *et al.*，2001；Reed *et al.*，2001；De la Poza *et al.*，2005）。Cry1Ab×Vip3A杂交棉田中绿草蛉丰度降低（Dively，2005），而Cry1Ab玉米田中的情况却不一样（De la Poza *et al.*，2005）。对瓢虫科进行的大部分田间试验表明，Bt作物不产生影响或产生不一致的影响。不过也有例外的情况，如Cry1Ab玉米和Cry3Aa马铃薯田中，瓢虫的数量增多（Musser 和 Shelton，2003；Pilcher *et al.*，2005），Cry1Ac棉花和Cry3Bb1玉米田中瓢虫数量减少（Men *et al.*，2004；Bhatti *et al.*，2005b）。在Cry1Ab玉米田中一些膜翅目寄生蜂的丰度降低（Dively，2005；Pilcher *et al.*，2005）。不过，有时只将Bt处理与杀虫剂处理的常规作物（以及相关对照）进行比较，因此没有研究转Bt基因作物的特定影响，而只是研究了相比于化学农药的特定影响（如 Riddick *et al.*，2000；Head *et al.*，2005；Torres 和 Ruberson，2005）。以上记录天敌密度变化通常是由于猎物的间接影响或植物介导的间接影响。就田间测试而言，重要的是要意识到昆虫的丰度变化很大，且受很多因素的影响。因此，试验设计和样本大小是获得必要统计检验能力的关键因素，这样测定潜在影响的能力才会高（Marvier，2002）。

已知Bt玉米品系176（Cry1Ab）的花粉可在田间对帝王蝶和黑凤蝶产生不良影响（Stanley-Horn *et al.*，2001；Zangerl *et al.*，2001）。虽然Bt176不再在美国种植，但却在欧洲进行了登记，如2003年西班牙的种植面积为32 000英亩（Lumbierres *et al.*，2004）。在美国，有人向Narure杂志写了一篇研究通讯，说明帝王蝶可能对Bt玉米花粉敏感，并在此后进行了一系列研究，以分析它对帝王蝶的任何潜在影响（Losey *et al.*，

1999)。很多团队启动了多项研究（如 Hellmich *et al.*，2001；Oberhauser *et al.*，2001；Pleasants *et al.*，2001；Sears *et al.*，2001；Stanley-Horn *et al.*，2001；Zangerl *et al.*，2001)。其中，用常规栽培措施管理的田间试验发现，MON810 和 Bt11 玉米花粉对帝王蝶 *Danaus plexippus* 和香芹黑凤蝶 *Papilio polyxenes* 都没有不良影响。与该结果相反，Dively 等（2004）表明在花药期，帝王蝶幼虫和成虫在自然条件下连续接触田间的 MON 810 和 Bt11 玉米花粉后，其存活、发育、体重和身体大小都受到不良影响。根据这些昆虫的敏感性和接触量可以推断，由于在美国只有少部分种群会接触到玉米田中落下的花粉，因此栽培表达 Cry1Ab 的玉米不会对帝王蝶造成大的风险（Mendelson *et al.*，2001；Dively *et al.*，2004)。在世界其他地区，如果农业用地和自然生境较近的整合在一起，非靶标鳞翅目昆虫对毒素的接触则需要更严格的监测。

只有少量关于 Bt 植物对土壤节肢动物的田间试验。一个关于苏云金芽孢杆菌 kurstaki 亚种（Dipel ES）对林区土壤动物影响的田间试验表明，其对蚯蚓、线蚓、甲螨、革螨和弹尾目昆虫没有影响（Beck *et al.*，2004)。Dively（2005）研究表明，在一个 3 年的试验中，转基因玉米（Cry1Ab ×Vip3A）对腐生性土壤节肢动物（包括跳虫和螨类）都没有不良影响。类似地，用于防治玉米螟的 Cry3Bb1 玉米对弹尾目昆虫、螨类、线虫和其他土壤中的无脊椎动物没有影响（Al-Deeb *et al.*，2003；Jasinski *et al.*，2003；Ahmad *et al.*，2005；Bhatti *et al.*，2005a；Bitzer *et al.*，2005)。Bhatti 等（2005a）在研究中观察到所测试的 14 类动物中只有 2～3 类动物受到了很小的影响，3 年中有 2 年出现 Bt 玉米小区中唇足纲的数量减少，1 年中出现隐翅虫科丰度降低，Bt 对双尾目的影响在各年间变化很大。在法国对 Bt 玉米（品系为 176）进行的田间测试中，在整个生长季节中，未见土壤节肢动物的多样性指数和行为受到显著的统计学影响（Candolfi *et al.*，2004)。

由于人们关注土壤中天然存在的以及 Bt 植物中残留的 δ-内毒素可能会在土壤中持续存在（见本节），因此进行了很多试验来评估毒素对非靶标生物（包括土壤微生物和大型生物）的影响。其中 Saxena 和 Stotzky（2001）首先报道了在 Cry1Ab 玉米种植 40 天后的土壤中或加有 Cry1Ab 玉米生物量的土壤中，蚯蚓、线虫、原生动物、真菌和细菌（包括放线菌）没有受到影响。在蚯蚓肠道中发现了这一毒素，但是将蚯蚓放到非 Bt 土壤中 2～3 天后毒素消失。蚯蚓的这一研究结果与对苏云金芽孢杆菌亚种 kurstaki 进行的 7 年田间试验结果相吻合，该研究中，微生物 Bt 在 3 种蚯蚓和 1 种大蚊科幼虫中萌发，但是对这些生物没有造成有害的影响（Hendriksen 和 Hansen，2002)。蚯蚓以及其他土壤微生物似乎为苏云金芽孢杆菌很多亚种提供了生长环境，使苏云金芽孢杆菌在世界各地的土壤中广泛分布。在一个为期 200 天的实验室试验和田间试验中，未发现取食了 Bt 玉米叶片的蚯蚓死亡，尽管 200 天后一些成虫出现了无法解释的体重减轻现象，结果可见第 74 和 73 段（Zwahlen *et al.*，2003a)。用 Dipel176 对微环境进行的一项实验室研究表明，在田间条件下，Bt 对非靶标微生物群落不可能产生显著影响（Visser *et al.*，1994)。除了 2001 年 Saxena 和 Stotzky 发表的论文之外，近来发表的大量论文都没有发现 Bt 植物对微生物群落造成显著影响（Dunfield 和 Germida，2004；Motavalli et al.，2004；Devare *et al.*，2004)，或影响很小（Blackwood 和 Buyer，2004)。

应当指出，即使可以充分评估土壤微生物群落改变所带来的所有风险，但难度也会很

大。土壤微生物群落因土壤类型、温度、湿度、植物长势、营养、pH 而变化极大，也会因在不同地点和同一样点的不同距离（即使是很小的距离）而显著不同。土壤食物网络结构因气候条件和地理条件而异（Neher，1999）。栽培和种植单一农作物对土壤微生物群落产生了严重影响，并且微生物种群数量极富弹性。即使采用化学熏蒸（用溴化钾）之后，土壤微生物还是可以快速再生。测定微生物介导的反应时常用的一个方法就是评价土壤种群活性，但并不能完全了解这些改变所产生的影响。近来一篇关于土壤微生物多样性研究现状的综述（Nannipieri *et al.*，2003）指出，由于微生物活性的天然冗余，任何一组微生物的降低通常会使其他微生物取代之前微生物的功能。文中也提醒，如果没有考虑这些限制因素对微生物群落的影响，则我们在进行群落分析时应当慎用新方法；某些改变到底是不良影响还是有利影响，这些都难以界定，可能经常不会有肯定的答案；同时也提醒不要将这些结果推广为普遍性的结论。

4. 其他生态学方面的问题

（1）杂草种群生物防治效率的丧失

具有杂草特征的农作物野生近缘种如果从其近缘作物中获得并表达了 δ—内毒素基因，则野生近缘种可以作为生物防治因子而释放，用于防御昆虫。由于杂草生物防治的昆虫有可能也是该农作物的害虫，因此不可能将野生近缘种昆虫作为杂草生物防治的因子。不过，天然发生的农作物害虫可能会减轻对近缘杂草的影响。在向日葵和油菜的研究中已经明确了杂草发生的增加潜力。向日葵 *Helianthus annuus* 的野生种在农业设施中可能是一种杂草。在美国西部和中西部，向日葵栽培种经常与野生向日葵杂交。Snow 等（2003）对野生向日葵中的 *cry1Ac* 基因进行了研究。在 2 个试验点中，鳞翅目害虫对转基因植物的危害较对照植物明显降低，而几种象鼻虫和实蝇却不受影响。这些结果表明：在内布拉斯加州的田间试验点中，植食性昆虫的降低（鳞翅目昆虫而非其他植食性昆虫）会使每株转基因植株产生的种子比非转基因对照平均多 55%。在科罗拉多州的趋势相似，但结果不显著（每植株所结种子多 14%）。在一个温室试验中，转基因对繁殖力没有影响，说明这一影响与适合度成本无关。如果 Bt 向日葵投入商业应用，则作者预计 Bt 基因将扩散到野生种群和杂草种群中，并能限制这些植株上敏感种群的危害，而且如果这些植食性昆虫为常见昆虫的话，将会增加种子的产量。在其他试验中，已经表明 Bt 油菜可在实验室和田间与野生近缘种杂交。温室试验已经说明 Cry1Ac 可能会赋予适合度优势，但是尚未进行田间试验来证实这一说法（Halfhill et al.，2002；Vacher *et al.*，2004）。

异型杂交的潜力是评价这种风险的重要部分。如前所述，异型杂交的潜力具有很强的作物特异性和地区特异性，在相关文章中已有很好的介绍。如果在转基因种植的邻近区域发生转基因植物与野生近缘种之间的异型杂交，则可用各种基因工程或种植策略来降低或消除异型杂交的潜在影响。

（2）对濒危物种产生不良的潜在影响

风险评估中应当考虑毒素对非靶标生物尤其是濒危物种的风险。濒危物种所在地限制使用传统化学农药，这说明应当评价特异性更强的 δ-内毒素产生的潜在不良影响。测试表明 δ-内毒素具有相对的特异性，即他并不影响某一目昆虫的所有物种。表达 Cry1 或 Cry3 蛋白的植物对濒危鳞翅目或鞘翅目昆虫的影响是应主要考虑的事项，而且风险评估

应当考虑是否有可能接触的稀有物种或濒危物种。尽管对潜在影响的考虑主要集中在对农业生境的影响，但是也应考虑 Bt 毒素是否会通过花粉而转移至邻近生境中。特别地，像结构景观区域的情况更是如此，如欧洲部分地区，它们靠近自然保护区或生态敏感区，或这些地方本身就是自然保护区或生态敏感区的一部分。在美国，有 229 种鳞翅目害虫的幼虫取食的寄主植物与玉米有关（Losey *et al.*，2003）。Schmitz 等（2003）报道，德国有 7%的鳞翅目昆虫主要发生在耕地中，并且可能会接触到 Bt 玉米花粉。这一研究表明，在 97 种昆虫中有 39%是稀有物种或濒危物种。作者建议在欧盟实施一个相关的风险监控计划，将这些物种纳入监控范围内。Wolt 等（2005）建议根据性状、转基因作物的时空表达模式和受体对环境的影响，采用分阶段的方法进行全面的风险评估，从而确定是否有必要采用监控或缓解步骤。

在评估毒素对稀有物种或濒危物种的风险时，也需要考虑异型杂交的潜力。Bt 性状渗入到野生近缘种会大大增加物种对毒素的接触，并导致 Bt 性状向未控制的生境中扩散（Snow *et al.*，2003）。Letourneau 等（2003）列出了世界各地在棉花、油菜、水稻及其野生近缘种上取食的 502 种鳞翅目害虫，如果 Bt 植物逃逸或发生远交，则这些昆虫会接触毒素或可能处于危险中。

（3）效率丧失的潜在危险

迄今为止，对 Bt 具有抗性的鳞翅目害虫如玉米螟 *Ostrinia nubilalis* 或粉茎螟 *Sesamia nonagrioides* 尚未在欧洲大田中发现（Evans，2002；Bourguet *et al.*，2003；Farinós *et al.*，2004）。尽管室内测试表明，玉米螟种群可形成对 Cry1Ab 一定程度的耐药性（Huang *et al.*，2002），但是室内筛选和 F2 代筛选均没有产生高抗的玉米螟品系（Bourguet，2004）。不过，另一种鳞翅目害虫（小菜蛾 *Plutella xylostella*）在美国已经发展了对 Bt 毒素的抗性（Tabashnik *et al.*，2003）。连续几年大规模栽培 Bt 作物可增加其对害虫的选择压力，从而导致抗性的发展（Fox，2003）。这可能会产生几种后果，其中包括需要用另一种植物检疫措施（包括使用杀虫剂但不用 Bt 毒素）来防治害虫。由于在田间条件下，在几年的栽培中均未有产生抗性的报道，因此出现这种情况的可能性很小。不过，很难预测害虫种群的未来反应。因此，从长效的角度考虑，我们应在 Bt 作物栽培过程中监控潜在靶标害虫的抗性发展。目前人们已经建立了一些方法用来阻止或延缓田间害虫抗性的发展（Williams *et al.*，1992；Rajamohan *et al.*，1998；Matten，1998；Pittendrigh *et al.*，2004）。

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由公司支持的产品开发研究实例

由于当时其他国家没有应用识别编号，下列表格参考了用美国识别编号标记的试验。USEPA 审查人员认为不充分的研究不在表格之列。本文提交的研究部分针对的是不予登记或登记正被驳回的产品。不过，作为对 δ-内毒素的总体介绍，这些研究仍然很有用。这些研究用一个主记录识别（Master Record Identification，MRID）编号来区别，其中 MRID 用于确定这些研究在档案系统中的位置。这些信息向公众开放。获得这一信息的最好方法是通过信息自由局（Freedom of Information Office），地址：HQ FOIA Operations Staff，United States Environmental Protection Agency，1200 Pennsylvania Avenue (1105A)，Washington，DC 20460，(202) 564—7333；E-mail：hq. foia@epa. gov，网页：http：//www. epa. gov/foia/。

表 1　为了申请 Cry1Ab 产品登记而提交给美国环境保护局（USEPA）审阅的研究

分　析	微生物毒素（MT）或植物毒素（PT）	结　果	USEPA MRID 编号
微生物毒素与植物毒素的等同性（＊＋ELISA）	在大肠杆菌 *E. coli* 中表达的 MT ＋ PT	MT 和 PT 相等（6 444）	433972 - 02
微生物毒素与植物毒素的等同性（＊）	苏云金杆菌中的 MT ＋ PT	MT 和 PT 相等（6 430）	435332 - 03
对小鼠的急性口服毒性		无影响浓度值＞4 000 mg/kg	434680 - 01
对小鼠的急性口服毒性	MT	无影响浓度值＞3 280 mg/kg	433236 - 08
对小鼠的急性口服毒性	PT	无影响浓度值＞5 050 mg/kg	434175 - 02
可消化性	MT 和 PT	被胃蛋白酶降解	433236 - 06
可消化性＋热稳定性	MT	被胃液降解，但是不被肠液降解，在加工的玉米粕和棉子粕中失活	434392 - 01
对鹌鹑的急性口服毒性	玉米粕中的 PT	无影响浓度值＞100 000 ppm 玉米粒	435332 - 05
对鹌鹑的急性口服毒性	PT	无影响浓度值＞2 000 mg/kg	433236 - 09
对蜜蜂成虫的毒性	MT	无影响浓度值＞20 ppm	434392 - 03
对蜜蜂幼虫的毒性	MT	无影响浓度值＞20 ppm	434392 - 02
对蜜蜂幼虫的毒性	花粉中的 PT	无影响	434157 - 03
对瓢虫的毒性	MT	无影响浓度值＞20 ppm	434680 - 05
对瓢虫的毒性	花粉中的 PT	无影响	433396 - 02
对绿草蛉幼虫的毒性	MT	无影响浓度值＞20 ppm	434680 - 03
对寄生蜂的毒性	MT	无影响浓度值＞20 ppm	434680 - 05
对水蚤的毒性	MT	无影响浓度值＞150 mg/l	433236 - 10

（续）

分　析	微生物毒素（MT）或植物毒素（PT）	结　果	USEPA MRID 编号
对弹尾目中 2 个物种的毒性	MT	无影响浓度值>200 ppm	439416 - 01
对弹尾目昆虫的毒性	PT	LD_{50}为 40 mg/kg/土，观察不到影响的浓度值（NOEL）为 125 mg/kg/土	434635 - 01
对弹尾目昆虫的慢性毒性	PT	无影响浓度值包括繁殖>50%饲料	442715 - 01
对鲶鱼的毒性	玉米粕中的 PT	100%饲料时无影响	438879 - 01
对蚯蚓的毒性	MT	无毒	433396 - 01
对蚯蚓的毒性	MT	无毒性浓度>200 ppm	438879 - 02

注：* 十二烷基磺酸钠—聚丙烯酰铵凝胶电泳（SDS-PAGE）、Western 印迹、N-末端氨基酸序列测定、糖基化和生物活性。

表 2　为了申请 Cry1Ac 产品登记而提交给 USEPA 审阅的研究

分　析	微生物毒素（MT）或植物毒素（PT）	结　果	USEPA MRID 编号
微生物毒素与植物毒素的等同性（*）	在大肠杆菌 *E. coli* 中表达的 MT+PT	MT 和 PT 相等（6 445）	431452 - 02
对小鼠的急性口服毒性	MT	无影响浓度值>4 200 mg/kg	431452 - 13
对小鼠的急性口服毒性	MT	无影响浓度值>5 000 mg/kg	439995 - 01
可消化性		被胃蛋白酶降解	439995 - 03
可消化性+热稳定性	饲料中的 PT	被胃液降解，在加工的棉子粕中失活	431452 - 14
对鹌鹑的急性口服毒性	花粉中的 PT	无影响浓度值>10 000 ppm	431452 - 11
对烟草天蛾 *Manduca Sexta* 的毒性	MT	无影响	439995 - 11
对寄生蜂的毒性	MT	无影响浓度值>花粉和花蜜中浓度的 10 000 倍	431452 - 08
对蜜蜂成虫的毒性	MT	无影响浓度值>花粉和花蜜中浓度的 10 000倍	431452 - 07
对蜜蜂幼虫的毒性	MT	无影响浓度值>花粉和花蜜中浓度的 10 000倍	431452 - 06
对瓢虫的毒性	MT	无影响浓度值>花粉和花蜜中浓度的 10 000倍	431452 - 09
对绿草蛉幼虫的毒性	MT	无影响浓度值>花粉和花蜜中浓度的 10 000倍	431452 - 10
对绿草蛉幼虫的毒性	MT	无影响浓度值>20 ppm	434680 - 03
对弹尾目昆虫的毒性	PT	无影响浓度值>8.0 g/kg	439995 - 12，- 63
对弹尾目昆虫的毒性	MT	无影响浓度值>0.1mg/kg	439416 - 01

表 3　为了申请 Cry3A 产品登记而提交给 USEPA 审阅的研究

分　析	微生物毒素（MT）或植物毒素（PT）	结　果	USEPA MRID 编号
微生物毒素与植物毒素的等同性（*）	在大肠杆菌 *E. coli* 中表达的 MT+PT	MT 和 PT 相等（6 432）	429322 - 03，- 04，- 05，- 06
对小鼠的急性口服毒性	MT	无影响浓度值>5 220 mg/kg	429322 - 17
可消化性		被胃液降解但是不被肠液降解	429322 - 18
对鹌鹑的急性口服毒性	饲料中的 PT	无影响浓度值>50 000 ppm	429322 - 14 429322 - 15
对寄生蜂的浓度	PT	无影响	429322 - 11
对蜜蜂幼虫的毒性	MT	无影响浓度值>100 ppm	441247 - 02
对蜜蜂幼虫的毒性	PT	无毒	429322 - 09
对瓢虫的毒性	PT	无影响	429322 - 12
对绿草蛉幼虫的毒性	PT	无影响	429322 - 13
对弹尾目昆虫 2 个种的毒素	MT	在 200 ppm 时无影响	439416 - 01
对蚯蚓的毒性	MT	无影响浓度值>100mg/kg 土	441247 - 01

表 4　为了申请 Cry9C 产品登记而提交给 USEPA 审阅的研究

分　析	微生物毒素（MT）或植物毒素（PT）	结　果	USEPA MRID 编号
微生物毒素与植物毒素的等同性（*）	在大肠杆菌 *E. coli* 中表达的 MT+PT	MT 和 PT 相等（6 466）	443844 - 01
对小鼠的急性口服毒性	MT	无影响浓度值>3 760 mg/kg	442581 - 07
可消化性+热稳定性	MT	不被胃液降解，受热（90℃，10 min）不降解	442581 - 08
同源性		与 SWISS 数据库中的过敏蛋白序列无同源性	442581 - 09 443844 - 04
对鹌鹑的急性毒性	PT	无影响浓度值>58 μg/l 饲料	442581 - 14
对蜜蜂成虫的毒性	花粉中的 PT	无影响浓度值>5.8 μg/l 饲料	443843 - 02
对瓢虫的毒性	花粉中的 PT	无影响浓度值>0.36 μg/l 饲料	442581 - 11
对大型蚤的毒性	花粉中的 PT	无影响浓度值>0.36 μg/l 饲料	442581 - 12
对弹尾目昆虫的毒性	MT	无影响浓度值>20 mg/kg 土	442581 - 10
对弹尾目昆虫的毒性	PT	无影响浓度值>180 mg/kg 土	442581 - 10
对蚯蚓的毒性	PT	无影响浓度值>1.84 mg/kg 土	442581 - 13
对非靶标有益昆虫的田间试验	PT	3 年以上的试验中 Bt 和 Bt 田中昆虫的类型和数量无差异	442581 - 15
昆虫的寄主范围研究	花粉中的 MT，PT，PT	对 Cry9C 敏感的昆虫有：欧洲玉米螟、烟青虫、小菜蛾；不敏感的昆虫有：亚洲玉米螟	442581 - 06

表 5 为了申请 Cry1F 产品登记而提交给 USEPA 审阅的研究

分 析	微生物毒素（MT）或植物毒素（PT）	结 果	USEPA MRID 编号
微生物毒素和植物毒素的等同性（＊）	大肠杆菌 *E.coli* 中表达的 MT＋PT	MT 和 PT 相等	450201 - 03 447149 - 03
对小鼠的急性口服毒性	MT	无影响浓度值＞5 050 mg/kg	446911 - 01 450201 - 18
可消化性	MT	被胃蛋白酶降解	447149 - 03
糖基化	MT＋PT	不发生糖基化	447149 - 03
热稳定性	MT	在 75℃及 75℃以上不稳定	452748 - 01
与已知过敏原氨基酸序列的相似性		Cry1F 与已知过敏原不存在连续 8 个氨基酸序列的同源性	449717 - 01
对鹌鹑的急性口服毒性	玉米粕中的 PT	无影响浓度值＞100 000ppm	450201 - 12
对寄生蜂的毒性	花粉中的 PT	无影响浓度值＞320 ppm，是花粉的浓度 10 倍	450201 - 11 453078 - 03
对蜜蜂幼虫的毒性	花粉中的 PT	在幼虫至成虫的整个发育过程中，LC_{50}＞640 ng/头	450415 - 03 453078 - 05
对瓢虫的毒性	花粉中的 PT	无影响浓度值＞480 ppm，是花粉浓度的 15 倍	450201 - 10 453078 - 02
对绿草蛉幼虫的毒性	花粉中的 PT	无影响浓度值＞480 ppm，是花粉浓度的 15 倍	450201 - 09 453078 - 01
对弹尾目昆虫的毒性	MT	无影响浓度值＞12.5 mg/kg 土	450201 - 07
对大型蚤 *Daphnia magna* 的毒性	花粉的 PT	无影响浓度值＞100mg/l	450201 - 08
对蚯蚓的毒性	MT	无影响浓度值＞2.26 mg/kg 干土	450201 - 06 453078 - 04
对帝王蝶幼虫的毒性	花粉中的 PT	LC_{50}＞10 000 ng/ml；无影响浓度值＜10 000 ng/ml；在最高测试剂量中可见生长受到一定的抑制	451311 - 02
田间调查以下昆虫种群数量的影响：瓢虫、捕食性、褐草蛉和绿草蛉、小花蝽、猎蝽、姬蝽、寄生蜂、豆娘、蜻蜓、蜘蛛	PT	肉眼观察未见显著差异，只是 Bt 玉米中瓢虫、花蝽和蜘蛛的数量比非转基因玉米中数量多 对粘虫的数量没有显著差异，只是 Bt 玉米中寄生蜂和花蝽的数量多	450201 - 13

表 6 为了申请 Cry2Ab2 产品登记而提交给 USEPA 审阅的研究

分 析	微生物毒素（MT）或植物毒素（PT）	结 果	USEPA MRID 编号
微生物毒素与植物毒素的等同性（＊）	大肠杆菌 *E.coli* 中表达的 MT＋PT	MT 和 PT 相等	449993 - 01 449394 - 03
小鼠急性口服毒性	MT	无影响浓度值＞1 450 mg/kg	449666 - 02

（续）

分　析	微生物毒素（MT）或植物毒素（PT）	结　果	USEPA MRID 编号
可消化性	MT	被模拟的胃酸降解	449666 - 03
与已知过敏原氨基序列的相似性以及热稳定性		Cry2Ab2 与已知过敏原不存在连续 8 个氨基酸序列的同源性，在 120℃以及以上温度下不稳定	449666 - 04 449666 - 05 442353 - 04
对鹌鹑的急性口服毒性	PT	无影响浓度值＞100 000 ppm	450863 - 16
对淡水鱼的毒性	PT 棉子粕	取食 Bt 棉子粕的 LC_{50}＞饲料的 20％	450863 - 18 453371 - 03
对蜜蜂成虫和幼虫的毒性	MT	在幼虫至成虫的发育过程中，无影响浓度值＞100mg/ml 幼虫	453371 - 02 450863 - 07 450863 - 08
对瓢虫的毒性	MT	无影响浓度值＞4 500 ppm	450863 - 11
对绿草蛉幼虫的毒性	MT	无影响浓度值＞1 100 ppm，为棉花中所观察浓度的 21.6 倍	450863 - 09
对弹尾目昆虫的毒性	PT 棉花叶片组织	无影响浓度值＞69.5 mg/g 饲料	450863 - 14
对蚯蚓的毒性	MT	无影响浓度值＞330 mg/kg 干土	450863 - 13

表 7　为了申请 Cry3Bb1 产品登记而提交给 USEPA 审阅的研究

分　析	微生物毒素（MT）或植物毒素（PT）	结　果	USEPA MRID 编号
微生物毒素与植物毒素的等同性（＊）	在大肠杆菌中表达的 MT＋PT	MT 和 PT 相等	451568 - 03 454240 - 04 454240 - 05 454240 - 10 454240 - 11 455382 - 01
对小鼠的急性毒性	MT	无影响值＞2 980 mg/kg	449043 - 06
对小鼠的急性毒性	MT	无影响值＞3 200 mg/kg	455382 - 02
对小鼠的急性毒性	MT	无影响值＞3 780 mg/kg	449043 - 05
在胃中的消化性	MT＋PT	被模拟的胃液降解	449043 - 07 454240 - 06 455382 - 03
在肠道中的消化性	MT	被模拟的肠液降解成不能进一步降解的小分子物质（Cry 蛋白通常抗胰蛋白酶）	455770 - 02
热稳定性	PT	在 240℃及以上温度不稳定	454240 - 07
与已知过敏原的氨基酸序列相似性		Cry2Ab2 与已知过敏原不存在 8 个连续氨基酸序列的同源性	449043 - 09 454240 - 08

（续）

分　析	微生物毒素（MT）或植物毒素（PT）	结　果	USEPA MRID 编号
与已知蛋白质毒素的氨基酸序列相似性		Cry2Ab2 与已知蛋白质毒素无氨基酸序列同源性	449043 - 08
对鹌鹑的急性口服毒性	PT 玉米粒	无影响浓度值＞70 000 ppm	449043 - 15
对淡水鱼的毒性	PT 玉米粒	食用 Bt 玉米的 LC_{50}＞35％ 饲料	449043 - 19
对大型蚤 *Daphnia magna* 的毒性	花粉中的 PT	无影响＞120mg 花粉/l	449043 - 18
对寄生蜂幼虫的毒性	MT	无影响浓度值＞400 ppm	449043 - 13
对蜜蜂幼虫的毒性	MT	在幼虫发育成成虫的整个过程，LC_{50} ＞1，790 ppm	449043 - 10
蜜蜂成虫	MT	LC_{50}＞360 μg/ml（在花粉中浓度的 20 倍）	449043 - 11
对绿草蛉幼虫的毒性	MT	LC_{50}＞8 000 ppm，田间接触浓度的 20 倍	449043 - 12
对瓢虫成虫的毒性	MT	LC_{50}＞8 000 ppm，植物中浓度的 20 倍	449043 - 14
对取食花粉的瓢虫幼虫的毒性	花粉中的 PT	LC_{50}＞93 μg/mg 花粉，幼虫发育成成虫	455382 - 04
对取食花粉的瓢虫成虫的毒性	花粉中的 PT	无影响浓度值 - *C. maculata* 了取食 50％花粉	453613 - 01
对取食花粉的瓢虫成虫的毒性	花粉中的 PT	无影响浓度值 - *H. convergens* 取食了 50％花粉	453613 - 02
弹尾目昆虫慢性食用后的毒性	叶组织中的 PT	LC_{50}＞872.5 μg（饲料中 50％ 的玉米叶）	449043 - 17
对蚯蚓的毒性	MT	LC_{50}＞570 mg/kg 干土	449043 - 16
对蚯蚓的毒性	MT	LC_{50}＞166.6 mg/kg 干土	457571 - 01
对取食花粉的帝王蝶幼虫的毒性	花粉中的 PT	无急性毒性或对发育无影响	455382 - 05
杀虫活性谱生测	MT	对鞘翅目 6 个科和鳞翅目 2 个种进行了检测，只有甲虫科的 2 种甲虫（玉米根虫和科罗拉多马铃薯甲虫）受到了影响	455328 - 07
两年的田间调查		非靶标无脊椎动物的丰度总体上无差异，对有益昆虫的影响比常规杀虫剂小	455382 - 06 457916 - 01

注：* 十二烷基磺酸钠－聚丙烯酰铵凝胶电泳（SDS-PAGE），Western 印迹，酶联免疫分析（ELISA）、N-末端氨基酸序列测定、液体芯片蛋白指纹飞行时间质谱系统（MALDI-TOF）分析蛋白消化、糖基化和生物活性。

表 8　为了申请 Cry34Ab1/Cry35Ab1 产品登记而提交给 USEPA 审阅的研究

分　析	微生物毒素或植物毒素	结　果	USEPA MRID 编号
微生物毒素与植物毒素的等同性（*）	在荧光假单胞菌 *Pseudomonas fluorescens* 中表达的 MT	MT 和 PT 相等	461239-05 461239-06
小鼠急性口服毒性	MT，Cry34Ab1	无影响浓度值>2 700 mg/kg 纯蛋白	452422-07
小鼠急性口服毒性	MT，Cry35Ab1	无影响浓度值>1 850mg/kg 纯蛋白	452422-08
小鼠急性口服毒性	MT，Cry34Ab1/Cry35Ab1 混合物	无影响浓度值分别为>482 mg/kg Cry34Ab1 纯蛋白和 1 520 mg/kg Cry35Ab1 纯蛋白	452422-09
在胃肠道中的消化性	MT	Cry34Ab1 和 Cry35Ab1 被模拟的胃肠液降解	452422-12 455845-02
热稳定性	MT	Cry34Ab1 和 Cry35Ab1 蛋白混合物置于 60℃，75℃ 和 90℃ 下 30 min 会失活	453584-01 455845-01 458086-01 458602-01
与已知过敏原氨基酸序列间的相似性		Cry34Ab1 和 Cry35Ab1 与已知过敏原之间不存在连续 8 个氨基酸的同源性	452422-05
与已知蛋白质毒素氨基酸序列间的相似性		Cry34Ab1 和 Cry35Ab1 与已知蛋白毒素无氨基酸序列相似性	465847-01
对淡水鱼的毒性	MT，Cry34Ab1/Cry35Ab1 混合物（**）	8 天急性毒性，无观察到的影响的浓度值（NOEC）>100mg/kg 饲料	457904-03
对大型蚤 *Daphnia magna* 的毒性	MT，Cry34Ab1/Cry35Ab1 混合物（**）	48 小时急性毒性 NOEC>100μg/mL	457904-04
对寄生蜂幼虫的毒性	MT，Cry34Ab1/Cry35Ab1 混合物（**）	11 天急性毒性 NOEC>280μg/mL 饲料	457904-05
对蜜蜂幼虫的毒性	MT，Cry34Ab1/Cry35Ab1 混合物（**）	6 天急性毒性 NOEC>5.6μg/幼虫	453407-01
对绿草蛉幼虫的毒性	MT，Cry34Ab1/Cry35Ab1 混合物（**）	10 天急性毒性，NOEC>280μg/g 饲料	457904-07
对异色瓢虫成虫的毒性	MT，Cry34Ab1/Cry35Ab1 混合物（**）	11 天急性毒性 NOEC>280μg/mL 饲料	452422-10
对取食花粉的十二星瓢虫幼虫的毒性	花粉中的 PT	7 天急性毒性，体重减轻 NOEC>58.52 μg/g 饲料	461239-12
慢性取食后的毒性对弹尾目昆虫的毒性	MT，Cry34Ab1/Cry35Ab1 混合物（**）	28 天急性毒性，观察不到对繁殖产生影响的浓度（NOEC）>12.7 mg/kg 饲料	457904-06
对蚯蚓的毒性	MT，Cry34Ab1/Cry35Ab1 混合物（**）	7 天和 14 天急性毒性 NOEC>76mg/kg 干土	453602-01

（续）

分 析	微生物毒素或植物毒素	结 果	USEPA MRID 编号
家禽取食后的毒性杀虫活性谱生测	饲料中玉米粒所具有的 PTMT，Cry34Ab1/Cry35Ab1 混合物	42d 饲喂试验没有与饲料相关的效应测试了 3 个目（鳞翅目、同翅目、鞘翅目）、4 个科（螟蛾科、金花虫科、蚜科和夜蛾科）的昆虫，发现只对 *Diabrotica* 属的幼虫产生影响	461239 - 11 457904 - 06
田间调查	PT	非靶标无脊椎动物的丰度总体上无差异	461239 - 14

注：* 十二烷基磺酸钠—聚丙烯酰铵凝胶电泳（SDS-PAGE）、Western 印迹、酶联免疫分析（ELISA）、N-末端氨基酸序列测定、液体芯片蛋白指纹飞行时间质谱系统（MALDI-TOF）分析蛋白消化、糖基化和生物活性。

**Cry34Ab1/Cry35Ab1 混合物的观察不到影响的浓度（NOECs）是 Cry34Ab1 和 Cry35Ab1 蛋白质浓度的和。

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