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生物安全:美国转基因作物的评价与管理

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摘 要 本篇综述侧重于介绍美国本土的转基因作物安全评价的过程和进展。回顾了安全评价程序的建立过程,从 1975 年阿西洛马(Asilomar)会议上关于重组 DNA 技术的问题,自 1984－1994 年间第一例转基因作物进行田间试验到评估作为食物和饲料商业化生产时,美国政府、学术界和产业界科学家之间的讨论。此外,一同回顾国际上的指南与美国的系统。整体过程还要考虑人类接触基因来源和表达蛋白是否有安全的或不安全的历史。食用蛋白安全性首要考虑因素为某些消费者是否对其过敏或者拥有转基因编码蛋白的 IgE 抗体,或者转基因食品会诱发乳糜泻,同时考虑表达蛋白的潜在毒性效果和对人或动物营养的影响。在美国,该过程与国际食品法典委员会的建议是一致的,它是基于科学的并应用合理的假设。迄今为止,没有证据证明在美国获得批准的转基因作物危害人或动物的健康。评价要考虑遗传和环境上的变化对植物新品种产物的影响,并坚持转基因植物食品和非转基因相似物种同样安全的评价原则。

关键词 转基因作物;食品安全评审;致敏性;毒性

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包括美国、中国等国际食品法典委员会(Codex Alimentarius Commission,世界卫生组织及联合国粮食和农业组织内部国际食品标准计划)(www.codexalimentarius.org)成员国在内的大多数国家对转基因生物或作物来源食品的安全性进行强制管理。国际食品法典委员会拥有包括欧盟在内的 185 个成员国和 224 个官方观察员(来自非成员国及非政府机构),并形成了对于食品安全和国际贸易等许多重要问题的指导意见。

仅有少数文献对转基因生物来源食品的安全评价整体流程进行过描述。针对研发者和监管者关于食品安全的主要指导意见出自食品法典委员会的联合文件(第 2 版)"现代生物技术来源的食品"[1]。在其第一章对基本原则进行了概述(CAC/GL44-2003),包括现代生物技术或基因修饰生物的定义和风险评价过程,该过程用于鉴定与新转基因生物相关的新危害或营养方面或安全性的问题,以及适当的风险管理程序。该文件随后的 3 个主要章节则明确了转基因植物、转基因动物和转基因微生物的安全性及营养品质方面的评价流程。这 3 类转基因生物的评价策略是非常相似的。食品的致敏性评估在每章的末尾以附件形式单独概述。潜在的毒性、乳糜泻和营养等同性的评估在每个文档的前面部分"实质等同性"章节讨论。法典为每一个国家建立自己的管理办法提供了指导,其目的就是使所有的法典成员国建立一个一致性的监管体系。除非有科学的依据,各成员国的监管体系之间不能存在差异。这些文件在 2001 至 2003 年间被制定,当前科学已有新的发展,这些将与公众进行讨论。同时一些新的评价步骤也将被讨论。本文着重探讨美国的评价过程。为了解一些反对转基因人士和组织针对转基因提出的问题和批评是否真正是目前没有被监管体制和研发者解决的合理安全问题,这些反对意见也将在本文中被探讨。

在美国、欧洲和中国,转基因食品的安全性问题一直是一些非政府组织(NGO)如绿色和平组织、地球之友、忧思科学家联盟以及电视和网络名人关注的焦点。我们必须认识到,消费者对于食物安全性的关心是很正常的,尤其是那些他们还没有完全了解的新食品或新技术。大多数消费者不明白致敏原

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的科学原理，也不知道过敏反应、毒素、营养和抗营养因子。他们也不明白作物的遗传多样性对于在不同的环境条件下进行生产的重要性[2]。许多消费者认为除非进行基因工程改造，所有的大豆都应该完全一样。其他领域的科学家也不明白粮食作物中蛋白质、油脂、碳水化合物以及各种代谢物的多样性和复杂性。植物育种家的主要工作是引入变异。如果我们考察基因工程的原理以及其带来的改变，显然生物技术相对于育种家用于培养新品种的自然变异或人工诱变，其引入的不确定性是最小的。我们是否可以向消费者解释真实的风险以及目前的评估过程是如何使风险最小化的吗？

转基因作物的反对者和制药业经常提到的一个重要的意见是，转基因产品的研发公司自己检测其安全性。这个意见看上去很合理，但涉及到了各个国家的整体法律框架、政府和经济结构。美国政府的科学家和其他许多国家一样，通常不会去检测大多数产品的安全性。值得一提的是，世界上没有任何一个政府拥有足够多的具有专业知识的科学家和足够多的资金在合理的时间内对所有的潜在产品执行相应的安全检测。许多重要产品的开发将会由于研发者必须等待政府的安全检测而被迫终止。在目前建立的监管体系内，大多数包括美国在内的国家只需要在其监管部门建立一支有资质的科学家队伍，他们可以根据相关的程序和指导进行严格的数据审查并做出决定。许多政府如美国政府，可以通过司法程序邀请学术专家进行咨询来协助进行评价。监管者也可以有效地同研发者进行沟通来告诉他们需要提供哪些额外的数据或检测，或者哪些问题还需要被回答以获得批准。

我对于孟山都公司这个主要的转基因作物研发者最为熟悉。他们拥有大约600名受过高等教育（学士、硕士和博士）的员工在涉及转基因审批的部门工作。这些科学家制定计划并开展安全和环境相关的研究，归档并对测试对象（植株、种子、DNA载体和蛋白）进行描述，开展测试，分析数据并提交报告给监管机构。他们需要在不同的环境下，有时候需在多个国家种植植物以完成田间测试和环境试验。监管相关的程序非常复杂，一个产品获得审批通常要花10～14年时间。类似孟山都这样的公司拥有专门的质量保证单位（quality assurance units，QAU），它向一个不同于研发和销售部门的管理部门进行汇报。QAU在开展研究前审查其方案、在向监管部门提交数据和报告前进行审阅，以确保研究的充分性和准确性。在一个产品被商业化种植前，公司的科学家们要评估海量数据并制作提交给多个政府的各类文档。大多转基因产品的种子在释放给农民前，需要获得主要贸易国家（澳大利亚、加拿大、中国、日本、韩国、美国）的行政审批。大宗商品或食品和饲料的国际贸易，只有在拥有材料和数据的研发者有能力和意愿确保及时协调地通过相关流程的情况下才能进行。在某些情况下，一些审批所需的研究特别是毒理学研究，需要通过合同在其他实验室进行。有些管理条例非常严格，要求这些特定的研究需要遵循由美国环保署（EPA）制定的良好实验室管理规范（good laboratory practices，GLP）。只有少数研发者的基础设施能够达到GLP要求。这些通过合同开展毒理学研究的公司可以满足审批的需要。相关研究可以由EPA审查。他们拥有非常严格的关于记录保存、职业道德和数据完整的规定。如果是政府来进行这些研究，谁又来审查他们数据并对其负责？谁又来保障这些研究可以及时被完成？

有些研究由一些进行学术研究的实验室完成，因为这些研究无论是研发者还是满足GLP的合同实验室都没有足够的专业水平来实施。我在内布拉斯加大学的实验室就为生物技术公司、非盈利农业机构以及开发新食品原料的食品公司进行过大量的致敏性研究（人血清IgE结合和生物信息学研究）。我们与临床医生合作，他们提供从愿意提供血清样本的过敏患者身上采集来的血清样本，以评价产品的安全性。我们建立了操作指南，开展研究，评估数据，撰写报告并保存相关研究记录。我们在与研发者在合同约定下开展研究，并遵守内布拉斯加大学和合作机构的道德标准。

我参与设计、开展和审查关于转基因作物和新食品添加剂的致敏性、毒性和营养品质和表现的相关研究已达17年。2001年，我参加加拿大温哥华举行的食品法典工作组会议并达成致敏性的指南[1]。我曾参与安全性研究，并审查过提交给美国、加拿大、阿根廷、巴西、欧盟、印度、韩国和中国台湾政府审查的转基因生物安全评价程序，我还对几百篇关于致敏性、毒理学和潜在基因水平转移的发表文献进行过审稿。在我的职业生涯内还未曾发现任何案例能证实人或动物在食用被批准的转基因作物后出现健康问题。我相信转基因生物的安全性审批

过程是非常可靠的[2-3]。

当然，我必须经历一个学习的过程才能达到充分了解转基因生物评价过程的水平，因为我天生是一个怀疑论者。本人的科学研究始于农业生物技术发展的初期。本文回顾了美国关于转基因作物的安全评价及管理的发展历史，包括一些之前被证实的食品安全相关的危害和风险，并介绍了在新转基因作物商品化释放之前安全性评估的流程。本文包括了一个典型案例，一个已被批准的转基因产品(Starlink，玉米)由于安全性数据的不确定(并非有危害性)而退出市场。

1　真实的与假想的食品风险

今天食用的很多食物我们在千百年前就开始食用了。很多作物的基因和确切的营养成分由于常规育种的改造，与原始品种相比已有很大的变化。很大程度上，大宗商品作物如小麦、水稻、玉米和大豆，还有很多的水果蔬菜与食物类似，人类安全地食用它们已有几个世纪。利用这些作物的经验指导监管者建立了一套安全评价流程，首先考虑人类是否有认识、接触或食用宿主植物(基因受体)和供体生物(被转化基因的来源)的经历。

在 20 世纪 70 年代中期，我刚刚取得生物学学士学位时曾是绿色和平、地球之友和忧思科学家联盟的积极成员。当时重组 DNA 技术的细节刚开始在大学课堂上讲授，我们认识到这个技术具有创造有用的重组细菌和植物的潜力。很多学生和教授对 DNA、RNA、核糖体和蛋白质合成的认识相对于 20 世纪 90 年代而言显得非常浅显，更不用说 2014 年的高校课堂所教授的知识。在 20 世纪 70 年代早期，保罗·伯格、沃尔特·吉尔伯特和弗雷德里克·桑格(后来都是诺贝尔化学奖的得主)开始讨论重组生物体潜在的(假设)危险，如致病病毒的 DNA 序列通过该技术导入细菌中。玛克辛·辛格等人在科学界呼吁建立安全标准。许多关注都集中到科尔等人提议的将构建到细菌质粒上的猿猴空泡病毒 40(SV40)的 DNA 片段转化到猴子的培养细胞中以研究基因功能[4]。作为回应，在美国科学院的敦促下，伯格等人组织的阿西洛马会议于 1975 年举行，讨论建立指南以保证安全。伯格和桑格综述了这一过程以及从那时起 20 年重组 DNA 工作的安全经验[5]。在指南建立的前一年，美国所有的重组 DNA 工作基本上都停止了。他们基于预期风险的周密考虑，要求所有从事基因工程研究的研究机构或公司建立各自的生物安全委员会，审查每一个新的重组 DNA 实验。主要重点是潜在的风险或者新 DNA 元件的安全性(基于 DNA 供体生物的行为模式和风险)。指南有助于确保利用该技术不会产生真正的危险生物。相对安全的克隆实验(生物安全等级 1 或 2)可以在一个典型的洁净实验室环境下进行，并对其的限制较少。在非常严格限制的极少数地方(生物安全等级 3 或 4)，可进行致命性和传染性病原体的重组实验(http://en.wikipedia.org/wiki/Biosafety_level)。

当然，转基因植物来源食物的安全性问题是不一样的。转基因植物没有传染性，转基因植物的食品安全性相对于微生物而言要低得多，与非转基因植物的风险没有区别。当然食物也有特殊的风险需要评估，如潜在的致敏原或者毒素从其他生物转移到粮食作物。很多假设的风险成为目前讨论的热点，而非基于我们已有的科学和安全知识去评估那些有限并且明确的风险。对于在作物(其本身含有 10 000 到 20 000 个内源基因，并且已被安全食用)中，添加了一个或几个基因或蛋白或新的代谢物而产生的新产品，其评估应重点放在基因来源、蛋白的特性和代谢物(如果导入蛋白是一个酶)的安全性。其他 10 000 多个基因和蛋白与目前作物已有的风险是一样的。而且，根据我们对其他食物的经验，新的基因和蛋白质呈现的风险类别是可以确定的。目前大多数非转基因作物食品都含有特定的致敏原的蛋白，一些还含有毒素(龙葵碱)或抗营养因子(胰蛋白酶抑制剂)。所以，对新蛋白的关注是评估其是否含有潜在的致敏性、毒性和抗营养特性。

一些被广泛种植的转基因作物的生产和消费历史已有近 20 年，比如含有苏云金芽胞杆菌来源的特殊蛋白的抗虫玉米即 Bt 玉米、转土壤细菌来源基因的耐除草剂大豆和抗病毒的木瓜。目前没有发现这些转基因作物有害的证据。生物技术改良的作物已经展现出很多优点，比如减少农药的施用或减少植物病原菌的影响(霉菌毒素)。大量转基因作物正在改善农业生产方式，如最大限度减少土壤退化以及能源和水的消耗。

有些人可能会说，正是因为对于转基因生物强烈的恐惧，引发了对适当监管研究的正常争论，从而促进可靠评价过程的建立。其他人则认为，研发者面对的监管要求已经过度，延缓了医学、工业和农业

科学的进步。真实情况可能位于两种极端观点之间。但是,同转基因研发者、贸易公司和食品公司的交流,以及对欧盟和其他国家的监管制度的考察,可以清楚发现过去10年(2004－2014)全球转基因的评价和审批进程明显拖累了研发进度,并导致全球性的贸易壁垒。由于贸易的国际化特性,农业公司必须等待很多年才能通过审批获许新产品进入全球主要市场。似乎所有国家的监管都变得越来越谨慎,他们害怕由于批准一个转基因作物、而这个转基因作物还没有在所有可能的情况下都被证明是绝对安全的而受到指责。预防性原则违背了美国食品和药物管理局(FDA)在1994年建立的政策。该政策认为所有食物的风险都是可以评估和管理的,安全的标准就是转基因作物来源的食物与传统作物来源的类似食物一样安全。而预防性原则只能接受零风险。

今天在科技文献中以“转基因”、“遗传修饰”、“基因工程”、“毒理学”、“生殖”、“癌症”作为检索词搜索,可找到很多针对转基因作物新的研究问题和设计,但这些研究问题和设计应该是基于作物或者是基因和基因产物信息的可检测的假设。极少有膳食蛋白可以影响生殖健康、导致癌症或增加大量自身免疫性疾病的发病率。那又为什么要对转基因作物的这些特性进行检测?

如今,活动家们如吉尔-埃里克·塞拉利尼、塔尔杰·特拉威克、范达娜·席娃、何美芸和杰弗里·史密斯,知名人士如奥普拉·温弗瑞、奥兹博士和崔永元,以及相信活动家们发表在网络、书籍、新闻媒体和电视上的关于转基因生物健康风险不恰当言论的消费者们,正在对监管者和政界人士施压。例如杰弗里·史密斯的网站,具有欺骗性的“负责任的科技研究所”(http://www.responsibletechnology.org/),声称非常多的人类疾病,包括自闭症、乳糜泻、食物过敏和癌症的发病率由于消费转基因作物而显著提高。他从观察数量很少和控制条件很差且未经验证的动物研究中得出结论,人类将会因食用转基因食物而患上各种复杂的疾病。史密斯先生还举办了一个要价150美元的“反转基因演讲家”的培训会。他没有发表任何可靠的经同行评议的科学研究论文以支持其说法,一般靠引用转基因产品增多且疾病同样增多的相关性(这类疾病是高度多样化和不明确原因引起的)。事实上这些相关性与转基因食物引入食物链并没有关系。然而,许多受过高等教育的人认同史密斯和其他的活动家的观点,并拒绝深入了解那些已经公开和发表过的、具有良好科学性的、证明批准的转基因生物已经经过安全评价的科学研究。没有研究能够将食用抗虫玉米和乳糜泻、食物过敏、自闭症或癌症联系起来。如果将我们的生活和环境中的巧合描述为必然的因果关系,我们就应该停止航空旅行,关闭互联网,丢掉手机和电视,禁止加工食品、疫苗以及处方药。我们应该以100年前的生活方式生活,那个时候世界人口不到20亿,生活和现在完全不同,而且人均寿命更低。

考虑食物风险时,食物的遗传多样性会导致其风险是非常值得怀疑的。人类是杂食性动物,依赖高度多样性的食谱。我们现在食用的食品和1914年时差异很大,更不用说1514年,那时马铃薯、辣椒和番茄还没有由南美洲引入欧洲、印度和中国。如果某种食用植物与我们平时食用的品种相比只因有非常少的遗传差异而表现出显著不同的风险差异,那么我们需要非常复杂的检测方法。然而,没有依赖高度复杂的科学研究,我们人类几千年来在评估食品安全性方面已经做得非常好。2014年,美国和中国延长的人均寿命、相对低的新生儿的死亡率和基础健康数据都有力地证明了目前的转基因生物不太可能是有害的。我们应该把关注重点转向食品的真正风险并建立相关流程保证转基因作物食品与非转基因作物食品同样安全。

2 转基因生物安全评价的早期发展

20世纪80年代中期,我还没有意识到转基因作物需要安全性评价以评估其食品安全性。我对于利用农杆菌介导的将功能DNA片段插入植物内的遗传转化过程知之甚少[6]。我在美国俄亥俄州立大学读博士时期学到了更多的生物技术相关知识。我克隆了牛乳铁蛋白基因的cDNA,并进行测序和表达,在校期间我必须学习和遵循学校生物安全审查委员会的评估。我必须回答基因来源、编码蛋白和质粒载体,以及接受克隆DNA的宿主细胞和生物体等相关问题。当进行老鼠和人类细胞因子的cDNA工作时,我在康奈尔大学以及随后的密歇根大学学习了免疫学,我也因此得到了进一步的训练。1997年,我作为一名监管转基因安全审查的科学家加入孟山都时,我不再相信绿色和平组织以及其他机构关于转基因作物的假想风险,以及他们声称的没有任何安全评价。我退出了这些组织。加入孟山

都 2 个月，我被安排建立一个动物模型，评估一个棉花转基因事件种子的潜在致敏性风险。这些测试都是新的和前所未有的，之前没有人证明啮齿类动物模型可以预测人类的潜在致敏性。然而，印度政府要求动物模型的致敏性测试。同一作物在美国被批准近 7 年后才被印度批准。印度在 2008 年调整监管方针与食品法典委员会指导意见(2003)保持一致[1]，不再要求用动物模型来评估潜在风险。我在孟山都的工作使我熟悉美国和其他国家的监管流程，并学习评估潜在致敏性、毒性和营养等同性的科学知识。2004 年我被内布拉斯加大学聘任后，继续参与监管评估流程，在 2014 年我甚至更广泛地参与其中。然而我仍在学习目前的评价相关过程。

一篇公开发表的综述显示，学术界、企业和政府的科学家合作通过多次协商后制定一个有用的可预知风险的转基因作物安全评价流程。美国政府在 1986 年制定了一个包括 FDA、EPA 和美国农业部(USDA)在内的协调监管框架，以评价和监管转基因作物(科技政策办公室，1986；http://www.aphis.usda.gov/brs/fedregister/coordinated_framework.pdf)。此时离第一例转基因作物获批还有 8 年。一群学术界和企业界的科学家举办会议成立国际食品生物技术委员会(IFBC)并与国际生命科学学会(ILSI)合作，建立了一套风险评估指导意见并发表在毒理学和药理学监管条例(1990)12 卷的补充材料中[7]。IFBC-ILSI 卷由 28 个资深科学家和法律专家完成，包含基因修饰的方法、传统食物中不同的作物组分、微生物来源食品原料的安全评价、单个化学体的安全评价、食物整体及复杂复合体的安全评价，还有法律和监管方面的事宜。该卷的草稿在发表前，经过了来自 13 个国家的 150 位企业、政府和学术界专家的评审。其中的主要问题在一次公开的会议中由 120 名专家提出并讨论。IFBC-ILSI 文档提出了许多关键的评价步骤和是否需要进一步评价的决定，并讨论了美国的食品安全监管法律框架。他们支持美国政府关于转基因作物来源食品能够在现有的监管框架下有效监管的决定，因为他们发现产生新品种的过程(如基因枪或农杆菌介导的遗传转化)对潜在的安全性影响与传统育种方法并无不同。该小组总结认为，重点应放在对导入转基因生物的 DNA、蛋白质或任何新酶导致的新代谢产物的特征研究和安全评价。

3　美国关于转基因生物评价的监管流程

1992 年 FDA 根据美国联邦食品、药品和化妆品法案(FDA 联邦登记第 57 卷，第 104 号，案卷编号 92N-0139)发布了植物新品种(包括通过重组 DNA 技术获得的新品种)来源食品的安全评价流程的政策声明。1994－1995 年第一例获批的转基因作物依据安全评价流程进行了安全评价，尽管现在(2014)更复杂些，但是其流程是一致的。USDA 动植物卫生检疫局(APHIS)负责监管未获批的转基因事件的田间试验，通过一个审批系统来控制包括转基因生物、植物害虫和兽药产品。USDA 另外一个部门，食品安全检验局(FSIS)负责监管肉类和禽类产品的安全性。FDA 负责包括转基因作物安全评价、牛奶和乳制品在内的其他食品安全问题。EPA 则主要负责评估转基因植物整合的杀虫性(PIP)基因(例如携带编码苏云金芽胞杆菌晶体蛋白基因的植物即 Bt 植物，携带抗病毒基因的植物如抗李痘病毒的李子树)，以及化学除草剂和杀虫剂。EPA 和 FDA 遵循相同的食品安全方针。对于 PIP 审批的标准流程是与 FDA 进行咨商，并将完整的文件档案提交给 EPA。虽然与 FDA 咨商以及提交数据在理论上是“自愿的”，但如果未与 FDA 咨商且未提交完整的转基因作物潜在致敏性、毒性和营养功效的评价数据，则有可能因出现疑似有害而被强制召回和被采取法律行动。EPA 和 USDA 的要求是明确强制性的。EPA 和 FDA 都希望在产品进入市场前接受相似的食品安全的评价和测试。

3.1　FDA 针对转基因生物食品安全性的政策：与非转基因生物的类似品种一样安全

FDA 及其他国家如澳大利亚、巴西、加拿大、日本、荷兰和英国的政府监管机构是 2003 法典指南的主要编写者[1]。中国和美国都接受其作为整个法典系统的一部分。该流程包含评价的风险与非转基因生物来源食品是一样的，即大家都熟知的对健康有不良影响的因素：食物过敏性、食物毒素和不良的营养因素包括潜在抗营养因子的增加或者潜在的乳糜泻诱导蛋白(小麦及其近亲来源的麦麸)。研发者需要提供人类安全使用(HOSU)或接触基因源和蛋白或基因历史的文档信息，以及引起不良后果的信息。这些信息必须包括基因产物(蛋白或 RNA)的特征、被引入酶的代谢物和基于新转基因生物来源食物的

消费方式来确定转基因生物中的蛋白或代谢物的消费量。如果有证据表明基因供体有潜在风险的历史记录,那么还需要额外的测试。

FDA认为所有食物的一些内源组分对消费者都可能存在某些风险。有些风险可通过食物的储藏、加工(如烹饪)或食用限制来降低。如凝集素、蛋白酶抑制剂和豆类的淀粉酶抑制剂,可以通过食用前烹煮而失活。木薯在做成木薯粉食用前可通过浸渍和挤压以除去氢氰酸防止氰化物中毒。马铃薯通过品种选育确保它们只含有低浓度的龙葵素(一种较温和的毒素)。幼嫩、绿色的马铃薯不能食用,因为这个阶段的龙葵素含量很高。人类已经适应了这些食物并通过加工以确保安全。这些危害如果不妥善处理,将会对所有食用者产生影响。其他可能会影响到每个人的威胁来自于细菌、真菌或化学试剂的污染。

我们需要认识到摄取食物最常见也是最严重的危险来自于外源物质的污染。这些污染可能发生在农场里,也可能通过餐馆和家庭的储藏而产生。细菌、病毒、真菌、寄生虫和化学物质包括真菌毒素、重金属和杀虫剂都是常见的污染物。最严重的危险来自于细菌,包括大肠杆菌O157和其他产毒菌株、单增李斯特菌、沙门氏菌属、弯曲杆菌属和产气荚膜梭菌。美国疾控中心(CDC)和USDA食品安全检验局估计,2014年美国3.1亿人口中有约3 000例的死亡案例和约128 000的住院案例(www.foodsafety.gov/poisoning/causes)是微生物污染造成的。一些寄生虫也通常通过食物传播。弓形虫病由弓形虫引发,是美国最常见的食源性寄生虫病,导致人们需住院治疗甚至死亡。有些病毒通常也通过食物传播。诺如病毒在美国是诱发急性肠胃炎的最常见原因,其因接触不卫生的加工食品而发生,但很少导致死亡。甲型肝炎感染敏感个体如不经治疗可导致死亡。霉菌的污染很少在人类的食源性疾病中记录,偶尔会出现一些发霉谷类产生的霉菌毒素中毒事件[8]。但是,通常会因喂食含高浓度霉菌毒素的谷物,导致家禽或其他农业重要物种的严重疫情。霉菌毒素是小分子到中等分子质量的有机化合物,通常是多环化合物,不容易被部分人群或物种的肝脏解毒。有些引发毒性反应的物质是蛋白质,如肉毒杆菌产生的肉毒素,蓖麻籽里的蓖麻毒素。蓖麻毒素是已知的极少数能够影响哺乳动物的植物蛋白[9]。有趣的是,转基因玉米表达植物整合的杀虫剂如Cry1A,能够减少烟曲霉素的水平(一种霉菌毒素),从而减少了鸡、猪和牛的中毒风险。未来的转基因产品很可能会表达出一些特异性抗真菌和抗微生物的蛋白,从而进一步增强食品安全和粮食安全。

毒素和抗营养因子几乎影响每一位消费者,但一些危害只影响一小部分人群。特定的食物致敏原可能只影响不到1%的人群,但是会引起剧烈反应甚至死亡。小麦的麦麸(醇溶蛋白和谷蛋白)与乳糜泻高度相关,这是一种患病人群不到1.5%的慢性自身免疫性疾病。乳糜泻受遗传因素影响,总人口的25%可能会受乳糜泻影响,而且还有其他很多未知的诱发因素。食品安全评估的一个重点是评价和保障将基因转入转基因植物时,不会转入一个致敏原,或者是将乳糜泻的诱发谷蛋白从一个乳糜泻的源头转化到另一个。

3.2 认识包括非转基因作物中包括乳糜泻在内的食物过敏风险

食用食物最常见的内源性风险是由IgE介导的食物过敏[10]和细胞介导的乳糜泻[11-13]。食物过敏可能会不同程度地影响2%到6%的美国人。一个人通常会对1~5种食物过敏。致敏原对过敏者造成显著风险,但不影响非过敏的消费者。食物过敏性因疾病严重程度和食物组分的复杂性不同而复杂多变,因而个体的致敏源并非总是很清楚[14-15]。诊断食物过敏的方法在许多医疗机构都不一致,很少医生受过良好训练可以精确诊断食物过敏[16-17]。食物过敏是特异的,因为每个患者都产生结合食物某个或多个蛋白质的IgE抗体。在过敏反应中,个体合成的特异IgE抗体至少结合食物中一种含量相对丰富蛋白质的2个表位(IgE结合位点)。他们的IgE抗体结合粘膜肥大细胞和血液嗜碱性粒细胞表面上FcεR1受体。如果摄取含过敏蛋白的食物后,这种蛋白或蛋白片段被吸收并结合粘膜肥大细胞和血液嗜碱性粒细胞上的IgE,刺激细胞内产生信号。如果几分钟内有足够多的致敏原-IgE结合发生,将诱发粘膜肥大细胞和血液嗜碱性粒细胞释放组织胺和白三烯,从而导致血管渗漏和由血管神经性水肿和神经刺激引起的相关症状。很多人经历过相对温和的口腔瘙痒以及口腔和咽喉轻度肿胀(血管性水肿),有人得过荨麻疹。一些人经历过哮喘引起的喘息和呼吸困难,有人有过呕吐和腹泻。少数人有过低血压(血压下降)。过敏性休克是一种严重的危及

生命的全身性反应，包括低血压和呼吸困难，通常需要立即就医，注射肾上腺素或其他药物并接受输氧。在美国，每年约有 150～200 例因食物引发严重过敏反应而死亡的案例[18]。大多数死亡案例都是因为没有及时接受医治，包括立即注射肾上腺素。花生、一些坚果、牛奶和鸡蛋都是一些常见的引起致命过敏反应的食物[19]。一旦个体接触致敏原而诱发过敏，这种过敏反应将可能伴随一生。然而，年幼的孩子可能会在接触过敏食物产生初次反应后的 5～10 年形成免疫耐受而能耐受致敏食物（牛奶、大豆或鸡蛋）。

食物过敏的发病率只能估算。美国、欧洲和日本有最好的估算数据，这些国家中 1%到 2%，最多 10%的人群受食物过敏影响[20-21]。有些严重的危及生命过敏案例的频率没有很好地统计。但是已经明确的是，花生、一些坚果、牛奶和鸡蛋肯定比水果和蔬菜更易诱发严重过敏反应。包括美国在内的大多数国家都没有一套标准的食物过敏上报机制。流行病学家在美国疾控中心审查医院 1997－2007 年的各种来源的医院编号，估计大约每年有 31.7 万病例与食品过敏有关（2003－2006 年），其中超过 9 000 例有严重的反应[22]。这些过敏反应通常和花生、贝类甲壳类（虾）、坚果、牛奶、鸡蛋和鱼有关。

乳糜泻是一种受由遗传因素影响的自身免疫性疾病，其由特定小麦、大麦和黑麦麦麸（醇溶蛋白和谷蛋白）激活辅助性 T1 型 $CD4^+$ T 细胞导致的[23]。这是一种慢性疾病，导致小肠上部绒毛萎缩，并且是一种消耗性疾病，有时会导致特定的癌症和其他自身免疫疾病。其遗传性主要是由于组织相容性复合体（MHC）位点 HLA-DQ2.5 或 HLA DQ8 的异常蛋白序列造成[24]。25%以上的美国人有 HLA-DQ2.5 或 HLA DQ8 两个位点中的一个，但估计只有 1%不到的美国消费者明确被诊断为乳糜泻，这和欧洲的患病率相似[25]。中国乳糜泻患病率未知，不过最近一个研究表明该病比以往认为的要普遍[26]。患病率的不确定性是由精确诊断的复杂性造成的，取食麦麸过敏反应以内窥镜多点活组织检测作为黄金诊断标准，而子宫内膜或组织转谷氨酰胺酶特异抗体 HLA 分型测试中，近亲的诊断也会被作为有效证据[27]。在有 MHC 遗传性的乳糜泻患者中已经鉴定到刺激 Th1 $CD4^+$ T 细胞克隆的谷蛋白和麦醇溶蛋白的特定多肽[28-30]。乳糜泻患者控制疾病的唯一途径就是避免食用含小麦、大麦、黑麦和一些燕麦蛋白质的食品[31]。越来越多的消费者认为他们并不对麦麸过敏，然而发病方式并不一致，反应的机制也未知，在疾病的鉴定上肠胃科专家之间还存在一些分歧[32]。

3.3　食物致敏原是特异蛋白，而不是整个食物

通常人们认为食物过敏就是对整个食物有过敏反应（如牛奶、鸡蛋和花生）。但过去 20 多年的研究已经确认食物中的特定蛋白才是导致过敏的原因。国际免疫学协会联盟（IUIS）致敏原命名小组委员会（www.allergen.org）列出 12 个常见的致敏原蛋白家族。报告中最突出的以花生蛋白命名的主要致敏原是小分子质量醇溶蛋白（14～18 ku），包括 2S 白蛋白（Ara h 2 和 Ara h 6）和较高分子质量的主要种子存储蛋白 cupin 蛋白（50～75 ku），Ara h 1 和 Ara h 3。每种 cupin 蛋白都占种子总蛋白含量的 15%以上。若某人这 4 种蛋白任意一种对应的 IgE 处于高浓度，在食用花生后会诱发最危险的剧烈反应[33-34]。Ara h 2 和 6 蛋白以 4 个链内二硫键形成高度交联的小分子质量蛋白，可以抵抗胃蛋白酶的消化[35-36]。还有其他的一些蛋白被鉴定为花生致敏原，但是丰度都很低，并且/或者蛋白稳定性较低，被认为是次要致敏原。大多数有明确 IgE 介导的花生过敏反应患者一定拥有主要过敏蛋白的 IgE，而次要致敏原是否引起临床反应还不清楚。其他食物或花粉中的一些被鉴定的与同源蛋白类似的蛋白被认为是泛致敏原，这是因为一个物质的 IgE 抗体可能会与很多物种的同源蛋白结合。泛致敏原一般不会引起剧烈的过敏反应。花生中的泛致敏原包括抑丝蛋白（Ara h 5），致病相关蛋白-10 家族成员（Ara h 8.0101 和 Ara h 8.0201）和一个脂质转移蛋白（LTP）Ara h 9。两个防御蛋白 Ara h 12 和 Ara h 13 的序列被 IUIS 命名委员会认可，但还未发布，并且其引发过敏反应的频率和程度都还未知。包括杏仁、榛子、山核桃和核桃在内的坚果引发的过敏反应通常与 IgE 抗体识别 2S 白蛋白和 cupin 种子贮藏蛋白类似的蛋白有关。某些情况下，似乎坚果蛋白之间甚至与花生蛋白之间存在交叉反应，但很难从“从头敏化”（de novo sensitization）分离 IgE 交叉反应，这样的物质是共敏化和共反应的。当然，山核桃和核桃有非常近的亲缘关系，它们的致敏蛋白几乎一样，因此，无疑有交叉反应。

在过去的 25 年中，一些独立的与 IgE 结合过敏源蛋白，如来自食物、吸入源（花粉，屋尘螨和霉菌孢子）和皮肤接触（乳胶）或注射源（毒虫叮咬），已被研

究和鉴定。利用合适的过敏者血清公开证明的IgE结合蛋白序列发布于内布拉斯加-林肯大学食物过敏研究和资源项目管理的数据库中(www.AllergenOnline.org),以提供一个转基因生物安全评价的生物信息学工具。该数据库中的一些蛋白已通过过敏对象的皮肤点刺试验、嗜碱性粒细胞组织胺释放或嗜碱性粒细胞活化证明,会引起生物反应,这些蛋白被充分证明为致敏原。每个致敏原蛋白组被分类罗列在数据库中(www.AllergenOnline.org),并且对每个归类的过程进行说明。该数据库还提供序列比对算法以评价新的转基因生物或新的食物蛋白存在交叉反应的潜在风险。

尽管IgE介导的过敏反应存在遗传风险因素,但是目前还不清楚为什么人们对特定蛋白和食物变得过敏,而不是对这些基本上是无害的蛋白变得耐受。发达国家食物过敏的发病率在升高,但这并不能归咎于消费者发生了遗传学上的变化[37]。一些机理被提出,其中包括"卫生"假说(在环境中或胃肠道内缺乏某些类型的细菌),其解释了过敏(特异IgE的诱导)和耐受(IgE和致敏原的抑制),但没有哪个机理或假说适合解释每种情况[37]。很可能在儿童期开始摄取食物时,食物加工方式,因久坐室内的生活方式导致的维生素D缺乏和过少接触某些微生物或寄生虫等多种因素相互作用,共同导致过敏性疾病的增加。

乳糜泻是由一定量的来自小麦、大麦、黑麦和也有可能的燕麦的麦谷蛋白和醇溶蛋白,以及所有Pooideae亚家族成员引起的。为了提供一个食物风险评估工具,我们收集了已发现会诱发乳糜泻T细胞的1 016个多肽和58个蛋白,建立了一个专门的乳糜泻数据库以用于风险评估(www.allergenonline.org/celiachome.shtml)。我们也开发了生物信息学工具通过比对以帮助评价新的食物蛋白,标注出潜在的有可能对乳糜泻患者有风险的蛋白。

4 美国的转基因作物安全评价

4.1 安全使用历史(HOSU)

安全使用历史的评价范围涵盖基因来源、基因受体和基因的特异产物,包括通过相关文献确定与蛋白存在直接接触或与代谢物间接接触(如果该蛋白为酶类)的情况。该领域的资深科学家已经描述了如何进行适当的致敏性和毒性评价[38-39]。如果基因来源本身是一种常见的致敏原或有毒性,则与没有任何过敏和毒性记载的基因源比较,需要额外的测试。例如,花生和一些坚果(核桃、山核桃、杏仁和榛子)被认为是过敏的常见原因。如果一个基因来自一个常见过敏源,特异性血清IgE测试就需要执行,如同Nordlee等人对巴西坚果2S白蛋白的研究一样[39]。如果基因源是蓖麻、*Closteridium botunlinum*细菌或黄蜂,监管者可能会要求额外的毒性测试以确定该蛋白不是一种毒素。特异的测试需求由该风险的天然属性确定。如果来源生物具有神经毒性,神经毒性测试就需要执行。可识别的风险来源一般可以通过搜索相关文献得到,谷歌搜索有时会有用。如果来源物有非常明确的使用历史,并且该蛋白被证明在来源物中表达(例如坚果、水果或草本植物),来源物没有致敏性和毒性将帮助确定该蛋白不太可能有风险。然而,在许多情况下没有这样的安全使用历史,并不意味着额外的测试是必须的,只是会稍微少些对安全性的肯定。

通常情况下,如果有明确的基因来源,风险会非常明确和有限。苹果含有的2种蛋白,可能被认为是显著的致敏原,一个会在极少人群中引起严重过敏反应的非特异LTP和一个效应不大的常见交叉反应蛋白Mal d 1。Mal d 1蛋白同呼吸道致敏原Bet v 1家族有序列相似性,Bet v 1在桦树和相关树种的花粉中很常见。其他苹果蛋白被认为导致食物过敏的风险很低或者没有。花生有4个烈性的致敏原和一些额外的小致敏原。食品标签法根据致病率和致敏程度以区分食物过敏的风险级别。在美国、欧洲和日本,花生被认为是一种常见的且很重要的食品过敏源,任何加工食物,只要含有花生成分就必须标注。而苹果就不是常见的烈性过敏食物。来自花生基因的蛋白要比来自苹果基因的蛋白接受更为严格的风险评价。

杀虫晶体蛋白Cry1A、Cry2A和Cry3A来自苏云金芽胞杆菌。该物种的孢子被用作微生物农药已有70余年,并且没有任何证据证明它们在哺乳动物中会导致过敏或毒性反应。作为有机农药的安全使用史,尽管只是证明Bt毒素作为微生物杀虫剂在细菌中表达而不是在所有物种表达是安全的,但仍然为Bt毒素提供了保证。

研发人员需要提供基因来源生物或者是基因产品(如果有)安全使用历史的文献资料。这些证据还包括提供关于在该材料中的表达蛋白或其他基因产物在食物中出现过,以及这种食物的加工过程。

4.2 新蛋白和产品的特征鉴定

研发者必须对转基因过程中转化的 DNA 或 RNA 进行说明。这些说明还包括其他遗传元件的来源(启动子和终止子)。转化的方式也需要描述。基因拷贝数、基因整合和 DNA 的遗传稳定性同样需要确定。基因产物需要在受体植物通常使用的条件下进行定量分析。某些情况下，mRNA 在各个组织中的大小和积累也需要检测以保证其转录产物与预期相同。大多数情况下，一个基因编码一个蛋白。如果蛋白是酶，任何预期的和测定的代谢产物都需要说明。基因和产物的功能必须公开。DNA 和蛋白的序列也需要公布并且蛋白氨基酸序列必须与其他毒素和致敏原比对以进行评估。

4.3 潜在致敏性

由于食物过敏的重要性，FDA 重视阻止致敏原转化到转基因作物新食品源中。对于花生过敏的消费者而言，一个编码花生致敏原的基因转化到水稻或者玉米中是一个很大风险。这个已被先锋公司(Pioneer Hi-Bred)的经验证明，他们转化了巴西坚果的 2S 白蛋白至大豆中，以改善动物饲料品质。大豆含有丰富的蛋白质，但缺乏含硫氨基酸。巴西坚果中的 2S 白蛋白是一种富含蛋氨酸和半胱氨酸的小分子蛋白。先锋公司提交该产品的评审材料时曾与内布拉斯加大学史蒂夫·泰勒博士交流，他建议既然巴西坚果对许多消费者而言是一种致敏源，就应该评估转基因表达蛋白的潜在致敏性。在 1995 年，没有人知道巴西坚果中哪些是致敏原蛋白。直到 Nordlee 等的研究成果公布才知道巴西坚果中的 2S 白蛋白是一种很明显的重要致敏原[39]。这些结果公布以后，先锋公司停止开发该产品，没有提交评审材料。这些经验帮助验证了 1992 年 FDA 联邦注册局发布的评价流程的正确性，并有助于 Metcalfe 等人发布的转基因蛋白潜在致敏性评价过程[40]的成型，以及食品法典委员会于 2003 年发布的指南[1]。

食物过敏通常限定于特异性抗原介导的 IgE 抗体反应和任何免疫学课本上描述的机制。大多数的膳食蛋白刺激免疫系统从而对这些蛋白耐受。然而，对于那些容易过敏的人群，他们的 T 辅助细胞和 B 细胞可能因为 T 辅助 2 型细胞提供了细胞因子和细胞表面信号的混合物，建立起 IgE 免疫球蛋白。B 细胞分化为浆细胞或 B 记忆细胞，表达高水平蛋白特异性 IgE，结合粘膜或皮肤的肥大细胞和血液中嗜碱性粒细胞的 FcεRI 高亲和受体。当抗原被再次通过饮食吸收时，如果至少 2 个表位被结合，它将偶联 IgE 抗体并启动信号级联反应。如果几分钟之内发生足够量的偶联，肥大细胞或嗜碱性粒细胞将释放组织胺、白三烯和蛋白酶并引起血管渗漏和炎症。症状可能包括血管性水肿、荨麻疹、哮喘、呕吐、低血压(血压下降)，并在极少数情况下，因全身性过敏反应而死亡。由于 IgE 抗体是特异性的肽表位识别，症状是可以再现的，相同的抗原，相似的反应。通常过敏反应的敏感性被认为是终生的。很多膳食蛋白也会诱发 IgG 和 IgA 抗体，但这些都不是剧烈食物过敏的风险因素。B 细胞也需要 T 细胞的帮助合成这些免疫球蛋白，但是响应和信号都不同于 IgE。因此，致敏性评估的重点是 IgE 反应。

也存在 T 细胞介导对膳食蛋白的反应，主要的一个就是前面提到的麸质敏感性肠病或称乳糜泻。对转基因生物蛋白潜在诱发乳糜泻的评价是相对直接的，这将在后文中讨论。很少有膳食蛋白诱发肠炎综合征(FPIES)的案例，这种烈性反应主要与牛奶和大豆中的蛋白有关，但很少在水稻和燕麦或其他一些食物中出现[41]。如果没有特异蛋白被鉴定为引起过敏的因子，个体通常在取食引起反应的食物 3 到 5 年后变得耐受该食物。因此，此时不可能将该蛋白评估为 FPIES 的可能诱因。

有可靠的证据表明，食物过敏和乳糜泻的发病率在全球范围有增多的趋势，但是对发病率增多的幅度和原因都未知。部分增多的案例可能是消费者对于食物过敏的意识增加，以及医生对乳糜泻的意识和诊断提高。有很多关于发病率的误导，并且人们常常被误诊。很多报告的食物过敏病例经临床评估后被证明并不是食物过敏。

食物过敏主要的风险是急性的，在摄食过敏源食物几分钟到几个小时后就会发生。转基因作物的首要食品过敏风险是潜在转化了的已经在特异消费者中发生过敏反应的蛋白。如果受影响的个体食用了含致敏原的转基因作物，将很可能像他们摄食天然来源的致敏原一样发生剧烈反应。因此，对于转基因作物而言，首要任务是避免转化这种能够导致过敏的蛋白(任何种类的，接触、空气或食物)到作为食物的植物的其他品种中。

国际生命科学学会(ILSI)-过敏和免疫学学会和国际食品生物技术委员会组织了一些讨论，并发

表一系列供同行评阅的文献讨论转基因作物潜在的食品过敏风险。该文章发表于食品科学与营养特刊(第36卷,增刊,1996)。该小组成员包括生物技术发展和监管、或致敏原、过敏方面的资深专家。第一章解释了过敏、食物过敏、植物蛋白的生物学、作物遗传修饰的过程,并对当时已知的过敏食物进行了综述;中间两章内容介绍了指导建立基于科学的新蛋白过敏风险评估流程的基本背景信息[42-43];最后一章概述了确定一个外源基因表达蛋白是否会对消费者产生过敏风险的评估流程[40]。

Metcalfe等人提出的评价流程[40]与FDA的建议(1992)一致,包含将基因来源的致敏性作为评价起始的决策树流程图。然而,决策树与文字描述并非完全一致,并且一些事情还没有描述清楚。图1表示我根据文字[40]对于该决策树的理解。如果一个基因明确来自于一个过敏源(食物、呼吸道或接触),下一步则是获得14个过敏人类的血清并通过标准实验室测试方法检测结合转基因蛋白的IgE。如果发现的可供测试的过敏供体少于5个,则评估该蛋白对胃蛋白酶的稳定性。所有的蛋白,无论何种来源,都应该接受与已知致敏原、食物性致敏原和呼吸性致敏原(1995年)的序列比对[40]。他们推荐使用FASTA格式将蛋白和已知致敏原进行比对,寻找连续8个氨基酸一致匹配的区域。在实际应用中大多数生物信息学匹配只是简单的执行滑动的8个氨基酸"文字"的匹配。如果一个蛋白质匹配上一个致敏原,过敏患者的血清就需要用于结合测试。如果在正确诊断的过敏患者血清中的IgE结合了该蛋白,研发者就可能要终止其开发并取消审批材料的提交。但是,如果他们打算继续,监管机构将可能会要求转基因来源的食品加上标签以警告过敏消费者避免食用。在不确定IgE结合的情况下,过敏受体就需要接受该蛋白的皮肤点刺测试(SPT),如果结果都是阴性,他们将被要求在伦理小组的批准下进行一个双盲且有安慰剂作为对照的食物测试(DBPCFC)。此外,该蛋白会被检测酸性环境和蛋白酶稳定性,以其消失的时间进行评级来评价其消化率。该蛋白对应作物可能根据其常规的食品加工过程进行测试,以确定加工过程中是否会被变性。然而,加工稳定性只是用于了解过敏或毒性风险能否通过常规食物加工过程减轻,类似于通过烹煮,豆科植物的天然凝集素和蛋白酶抑制剂可以失活。如

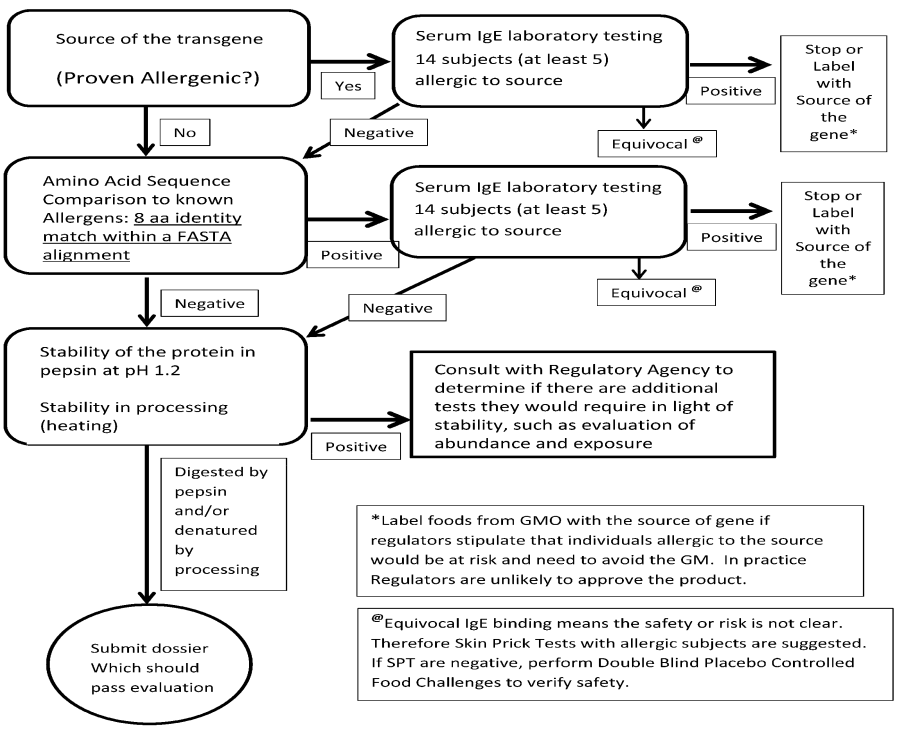


图1 对转基因蛋白的潜在致敏性评价

Fig.1 Assessment of the allergenic potential of GM proteins, adapted from Metcalfe et al, 1996 to more caaurately reflect the description in the text

果蛋白对胃蛋白酶稳定，监管机构将可能要求额外的测试。

美国批准的大多数转基因事件和新表达的蛋白符合 Metcalfe 等[40]和法典[1]提出的致敏性评估的风险最小原则。文献检索发现只有一种转基因作物产品不符合安全使用历史，并且在巴西坚果过敏症高危人群血清接受 IgE 结合测试中发现该蛋白可以结合 IgE[39]。该产品未被提交给监管机构，并被研发者(先锋公司)终止。据我所知，还没有接受来自致敏生物基因的产品被批准。因此，巴西坚果 2S 白蛋白是唯一一个可能对部分消费者具有较大过敏风险的蛋白。

4.4　匹配致敏原的生物信息学分析

除了评价基因来源，利用生物信息学搜索转基因蛋白与任何已知或可疑的致敏原之间的一致性匹配可能已成为最重要的鉴定潜在风险的工具，以及是否进行血清 IgE 检测的理由[38,44-45]。食品法典委员会[1]文件呼吁使用 FASTA 或 BLASTP 来利用转基因蛋白的氨基酸序列比对搜索一个已知的致敏原数据库。www.allergenonline.org 数据库是我知道的最全面的经同行评审的致敏原数据库。最重要的标准是与任何 80 个或以上的氨基酸的任何部分有大于 35%的一致性。致敏原在线数据库(www.allergenonline.org)于 2004 年在内布拉斯加大学的食物过敏研究和资源项目中建成，并进行了专家评审。这个网站每年更新，提供一个准确的数据库，以及使用生物信息学进行过敏风险评估的算法[44]。2014 年 1 月公布释放了第 14 版的数据库，包括来自代表了 290 个物种的 645 种蛋白分类群体的 1 706个序列。我认为，如许多已报道的文章所述，任何一段 80 个氨基酸序列中大于 35%的一致匹配搜索是相当保守的。很少有证据表明整体序列相似性不到 45%的全长蛋白质在体外有交叉反应。就由于交叉反应引起的过敏来说，很少有全长匹配小于 50%的蛋白质发生交叉反应[46]。自 1996 年以来，已经有很多科学咨询者和建议者建议“改进”转基因作物的致敏性评价。FAO/WHO[47]专家审查小组提出了一些没有生效的改进建议。FAO/WHO 推荐使用 FASTA 或 BLASTP 去比对识别匹配度大于 35%的任何 80 个或以上的氨基酸片段；搜索连续 6 个而不是 Metcalfe 等人提出的 8 个相邻的氨基酸短肽是否有匹配[40]。但这些预防原则尚未生效。这些建议在已发表的文献中进行了综述[3,38,45]。大量的研究表明，6 个氨基酸的一致性匹配搜索产生的假阳性比真实的阳性多得多。8 个氨基酸相似性比对较好，但仍没有一个高的预测价值。其他人也表明 80 个氨基酸的搜索过于保守；尽管使用这个标准我没发现很多假阳性的结果，也没有漏过任何可能交叉反应的蛋白对[45]。正如一些生物信息学家建议的，对于 80 个氨基酸的搜索，通过 FASTA 或 BLASTP 搜索最好的全长序列并且获得最佳匹配分数作为一个更可信的选择，但对于这个优化算法仍有一些分歧[48-50]。最近，欧洲食品安全机构(EFSA)[51]不再推荐搜索短的连续的一致性比对，并且鉴于 EFSA 和欧盟委员会[52]的关系，欧盟委员会也接受了这个建议。有一些例子表明，短的连续的匹配是偶然发生的(没有整体同源序列的证据)，这使得没必要的血清 IgE 检测变得必要[53]。在体外进行 IgE 结合实验也是有风险的，结果可能不明确，假阳性的结合结果会不准确地暗示一个蛋白可能是致敏原[54]。虽然 IgE 结合到 2 个非同源蛋白的一个单一小片段上，意味着在这种情况下有过敏风险的可能性很小，而一些监管机构可能会希望研发者继续研究，并可能要求进行人类的体内试验。

4.5　血清 IgE 检测

血清 IgE 的检测很少批准用于评价转基因蛋白的潜在致敏性。然而，如果血清 IgE 的检测被批准，则必须精心设计实验，并使用对于过敏性血清供体已知的致敏原来证实，并且参加试验的个体应有合适的过敏反应和 IgE 敏感。试验材料应包括纯化的转基因蛋白、纯化的致敏性靶标(如致敏原有匹配的序列)以及合适转基因材料的抽提物。此外，IgE 检测抗体的特异性需要验证，以及需要合适的封阻溶液阻止非特异性的抗体结合。在某些情况下，可能需要特定的抑制试验来检测其特异性。材料和方法设计中的关键因子已存在于发表的大量文献中[45,54-55]。如果蛋白质含有天冬酰胺连接的碳水化合物，植物可能会通过在天冬酰胺链多聚糖主干上添加 α-1,3 岩藻糖或者 β-1,2 木糖对蛋白进行修饰，因而可以与 IgE 结合，但是相应的个体没有临床反应[56-57]。这些结构现在已知为交叉反应的碳水化合物决定物(CCD)。如果有一个信号肽和 N 链接的 sequon(天冬酰胺-X-苏氨酸/丝氨酸)，这种蛋白被测试为存在 CCD。需要抑制试验来评价体外 IgE 结合的相关性。如果有体外 IgE 结合的证据，但又

希望继续进行产品研发,可利用嗜碱性粒细胞的激活或嗜碱性粒细胞组胺释放试验来检验结合作用的生物学相关性[58]。或者,利用高度特征性试验材料和愿意参加试验的知情受试者进行皮肤点刺试验(SPT)或双盲、使用安慰剂对照的食物测试。

4.6 潜在的从头敏化作用:胃蛋白酶稳定性和蛋白丰度

如果没有证据说明蛋白质可能是致敏原和缺乏生物信息学的匹配,那么就没有理由进行血清 IgE 试验。风险的概率很低而且没有处于风险的群体。关于致敏性唯一的问题是蛋白是否会重新致敏。Metcalfe 等人[40]建议,如果蛋白质在胃蛋白酶体外消化实验中是稳定的,而且含量丰富,则这个蛋白质很有可能是重要的食物致敏原。然而,这种相关性也并非很高。因为虽然许多主要食品致敏原是稳定的,或其部分片段是稳定的并且含量丰富[59],但是也有很多稳定且含量丰富的蛋白质并不导致过敏。胃蛋白酶稳定相关性的验证一般在 pH 值为 1.2 和 2.0 条件下进行,2001 年 FAO / WHO 建议使用这 2 种 pH 条件进行稳定性评价。不过,我们也没发现这 2 种情况有何显著差异[59]。EFSA 曾经建议使用更"生理的"pH 值(3.5)[57],但在这个条件下胃蛋白酶活性明显降低而且其预测性的价值并未被证实。FDA 一直接受的 pH 条件为 1.2 或 2。对于丰度,仍然没有一致意见,但是很明显的是,在植物中许多主要致敏性蛋白的丰度在食用部位大于蛋白含量的 1%[60]。

已发现大部分的转基因蛋白在 pH 为 1.2 或 2 时被胃蛋白酶迅速消化。然而,Bt 蛋白 Cry9C 最初由在比利时根特的植物遗传系统(PGS)公司导入到玉米中以抵抗玉米螟幼虫的危害,但被发现在胃蛋白酶中稳定。其产品被称为"StarLink"玉米。PGS 公司被 AgrEvo 购买,然后被 Aventis 作物科学公司收购,最后 Aventis 作物科学公司又被拜耳作物科学公司收购。作为食品应用的批准被暂停,因为蛋白质在胃蛋白酶消化实验中是稳定的(稍后介绍),而监管机构认为这有风险,最终可能会使某些人过敏,导致对 Cry9C 有过敏反应。1999 年,美国约有 12.2 万 hm^2 土地种植了 StarLink 玉米。由于一些意外,StarLink 玉米非法进入了一些人类的食品(玉米片和玉米卷)。反对转基因的 NGO 通过测试发现 StarLink 玉米进入了一些食品中,并通知了美国政府和新闻媒体。有趣的是,关于人们是否可能对这个蛋白质过敏的问题,使消费者过敏是需要一定时间的。没有迹象表明,人们之前接触过 Cry9C,因此过敏反应只能从接触受污染的玉米卷和玉米片开始。不过,在通告的 2 周内,有超过 100 位消费者抱怨在消费玉米卷壳或玉米片后有食物过敏反应。由于玉米是谷物中致敏原最少的作物之一,玉米粒中 Cry9C 的含量大约为 50 μg/g,这些谷粒的种植也仅仅只有 1 年,所以任何人对这种蛋白过敏都是不太可能的。然而,美国疾控中心还是调查了每一份消费者报告。那些声称有反应的个人可能含有食物致敏原,当被问及是否愿意提供血液样本,有 18 个人提供了[61]。这些人都没有 Cry9C 特异性的 IgE。由于谷物和玉米种子释放并未经批准在食品中使用,美国政府要求召回和监测。食品、食品原料和玉米种子被筛查,那些含 Cry9C 蛋白或基因的材料从市场被召回。一共花了 6 年或更久才完全剔除所有种子和粮食店中的 Cry9C 痕迹。粗略地估计,"剔除"的成本可能超过 5 亿美元。但我们应该记住,没有证据表明有人因接触 Cry9C 而受伤害。显然,来自转基因作物食品如 Starlink 玉米的过敏风险与诺如病毒、肝炎或大肠杆菌 O157 爆发的风险程度是不同的。我们可以得出这样的结论,监管机构的反应相对于 StarLink 事件的风险,并不相符。然而,对 StarLink 玉米的召回体现了能够从产品中剔除转基因作物的能力。这也表明如果有足够的理由,我们是可以从农业和食品系统中剔除转基因作物的。只是需要花费时间和大量的金钱而已。

另外一个产品是在其他的抗虫玉米中的 2 个蛋白(Cry34Ab2/Cry35Ab1),它们不像 Cry1Ab(玉米中)或者 CP4 EPSPS(耐除草剂大豆中)那样可以在胃蛋白酶中快速消化。这 2 个蛋白如研发者陶氏益农公司所报道的那样呈现一定的稳定性[62]。EPA 还是允许该产品进入市场,因为这 2 个蛋白在籽粒中的丰度低而且只是相对稳定。

目前在已被批准的转基因作物中表达的新蛋白在作物来源的食品原料中只是低水平的积累,通常小于 100 μg/g。我所知道的表达量最高的蛋白,包括耐除草剂大豆(孟山都公司)的某些品系中表达的 CP4EPSPS 蛋白高达 400 μg/g,以及在转基因玉米事件 3272 的种子中富集接近 2 000 μg/g 即 2 mg 每克种子干质量的淀粉酶 AMY797E 蛋白(先正达公司)(CERA 转基因作物数据库,2014;http://ce-

ra-gmc. org/ index. php/GMCropDatabase)。因此，所有的转基因蛋白积累的水平显著低于最重要的食物致敏原的浓度。

没有证据显示已获批的转基因作物会由于转基因蛋白造成过敏反应。为了确定对大豆过敏者的 IgE 是否可以结合 CP4EPSPS 酶，CP4EPSPS 酶导入大豆中使其具有草甘膦抗性。这不是一个监管审批需要的研究，而是作为管理需要的研究，只是看看是否有证据表明该产品进入市场多年后有没有过敏性。从欧洲和韩国收集了大豆过敏者的血清样品，使用了通用的检测方法和高度特异性的试验材料。该研究没有发现 IgE 结合纯化的 CP4EPSPS 蛋白或转基因大豆蛋白提取物的证据[55]。

4.7　对 IgE 介导的致敏性评价的潜在改进

FAO/WHO 机构推荐使用针对性人血清试验，来尝试确定一个与任何已知致敏原都不相似的蛋白可能存在的致敏或者交叉反应的风险。针对性的试验是指，在体外使用 50 个对转基因来源有关材料过敏个体的血清，来进行抗体 IgE 结合试验。如果基因来源于双子叶植物，那么一种或多种双子叶植物过敏者将被用于血清结合试验。对于细菌源蛋白一般不需要该试验，几乎没有人对细菌过敏。针对性血清试验的预测能力从来没有被证明过，它有违于我们对交叉反应知识的直觉。相对比较近缘(分类上“科”一级)的同源蛋白的体外试验，很少发生交叉反应以及临床反应。在实验室实验中，唯一具有广泛交叉反应的蛋白是抑丝蛋白(profilins)、PR-10 蛋白 (Bet v 1 同源物)、脂质转移蛋白与甲壳类和其他无脊椎动物来源的原肌球蛋白(tropomyosins)。这些通过生物信息学很容易识别。美国不认为针对性血清试验是新蛋白质评价的有用工具。

FAO/WHO 也推荐使用 2 种动物模型进行致敏试验，或者一种动物中的 2 种敏感途径来评估每个新蛋白的致敏性潜力。Ladics 等人的综述告诉我们，许多实验室都尝试过不同动物模型测试预测蛋白质的过敏性，没有一个试验可以预测各种程度的有效致敏性(从没有或轻度到强烈的过敏)[63]。有研究表明，评价过敏的机制和免疫疗法[64]，以及对过敏原进行初步评分还是有一些希望的[65-66]。几个研究测试了纯化的或者部分纯化的蛋白[67]，但是对新蛋白的过敏潜力或发病率的评分没有在人群中进行过验证[63]。美国不承认目前的动物模型对预测新蛋白的致敏性是有用的。

法典委员会的指南确实推荐使用 FASTA 或 BLASTP 搜索比对以确定任何部分的 80 个或更多的氨基酸序列一致性匹配大于 35% 的序列测试。法典委员会[1]还保留建议使用短的 6 或 8 氨基酸的匹配，但评价者必须科学地判断这一选择。美国监管机构目前要求使用 www. allergenonline. org 上的比较工具，或者通过 BLASTP 进行全长比较，以超过 80 个氨基酸大于 35% 的一致性作为标准，进行比较。他们似乎不在乎短的 8 个氨基酸匹配，但是大多数(或者所有)的研发者都提交了这一数据。

欧盟的管理条例[52]，很大程度是基于 EFSA 专家小组的评价过程，同时也包括一些未验证的试验如：对新蛋白的潜在佐剂性的评价；使用蛋白质组学考察常见致敏物种(如大豆、花生)的内源性致敏蛋白表达的潜在变化；使用更多的生理学 pH 条件(3.5)进行胃蛋白酶消化实验。然而目前这些方法均未证实会改进风险评价，且美国监管机构对其也不作要求。除非有合理的理由支持一个新的蛋白质可能是一种凝集素或有其他一些辅助性质，美国监管机构不要求额外的检验，如潜在的免疫辅佐。

4.8　乳糜泻

已发现乳糜泻相关的风险涉及某些来自小麦和近亲缘麦类的麦麸(醇溶蛋白和谷蛋白)。法典委员会[1]建议且美国政府也要求，如果小麦、大麦、黑麦或者燕麦来源的基因转入其他物种，比如玉米、大米或高粱需要进行评价。据我所知，目前还没有开发商向美国监管机构提交一个这样的潜在产品。虽然法典要求对来源于小麦或近缘种的蛋白进行评估，但他们没有对这一过程提供指导。我的实验室关注的问题是对乳糜泻以及涉及麦麸的已有知识，并开发一个乳糜泻数据库以提供一个快速鉴定潜在的危险性蛋白的生物信息学工具。为了开发这个工具，我们回顾了已发表的关于乳糜泻的科学信息，并描述如下。

对食用面包引起的吸收不良和腹泻的症状在约 2 000年以前的希腊医学著作中首次描述[68]。直到 1888 年，英国的一个内科医生才将这种食用含有麦类食品而遭受的肠道病痛命名为乳糜泻。现代医学并没有注意到这种现象，直到 1952 年，英国内科医生发表对这种由食用麦类引起消耗性的肠道疾病的相关描述。在 20 世纪 90 年代，胃肠病学家发展出了内窥镜检查和抗体检测的方法，证实乳糜泻患者携带的抗体能与小肠内的结缔组织结合，麦麸来源

的小麦多肽与主要组织相容性抗原受体结合激活患者的T细胞。近年来的研究发现了许多小麦、大麦和黑麦谷物中谷蛋白和醇溶蛋白的多肽，可以激活遗传上敏感个体的T细胞[30,69]。小麦、大麦和黑麦中蛋白是引起具有敏感性的MHC II类(MHC DQ 2.5和MHC DQ8)的少部分个体特异性T细胞反应的原因。由于许多这样的发现发生在20世纪90年代中期及以后，所以对小麦、大麦和黑麦中蛋白的评价才出现不久。此后，很多的研究鉴定出了结合正确的MHC以及激活乳糜泻患者中T细胞效应子的多肽序列。与此同时，Metcalfe等人[40]以及法典[1]的建议没有认识到生物信息学是有评估来自小麦亚家族蛋白的乳糜泻风险的预测能力。很明显的是，大量蛋白都被数据库确定为“有风险的”蛋白。2011年，我实验室的一个博士生Plaimein Amnuaycheewa，综述了50多篇发表的文献，这些文献使用来自乳糜泻患者的细胞样品鉴定涉及T细胞反应的多肽。我们开发了一个多肽的数据库，从来源于小麦或近缘种的蛋白中筛选出潜在危害性的多肽。我们建立了一个来源于小麦、大麦和黑麦的多肽数据库，这些多肽会引起T细胞应激反应或者肠道上皮细胞病理(www.allergenonline.org/celiachome.shtml)。这个数据库是www.AllergenOnline.org的一部分，它可以对转基因蛋白质引起的潜在IgE介导的致敏原性进行生物信息学评估。目前，这个数据库含的1 016个多肽都有公开发表的T细胞反应的证据，这些证据来源于与MHC II类DQ2.5或者DQ8相关的乳糜泻患者的细胞，或者对有乳糜泻患者的肠道上皮组织毒理学效应，或者乳糜泻患者肠绒毛组织的病理学。在数据库中，转基因作物中被转入蛋白质的氨基酸序列可与数据库中的多肽进行精确匹配的搜索，也可使用FASTA搜索比对已知的68个乳糜泻致病的全长蛋白质，如果至少100个氨基酸片段中有大于45%的一致性匹配则被认为可以潜在刺激乳糜泻发生。数据库中可能导致敏感个体发生乳糜泻的多肽和蛋白，总共参考了53篇文献。与致敏性评价类似，生物信息学的方法可以鉴定具有中等到明确风险的致病蛋白。如果需要将一种小麦亚家族蛋白导入到另一种作物，如水稻或者茄子，其氨基酸序列也应该利用这个数据库来筛选考虑其风险。如果发现阳性的匹配，这个蛋白应该使用细胞或乳糜泻患者进行试验来评估风险。这个试验应该是基于细胞的检测或者至少10个愿意测试的乳糜泻患者的食物检测，以确保其对乳糜泻患者即“处于风险的”消费者的风险最小化。我们认为生物信息学的标准是基于广泛的模拟来进行预测的，要求对于1 016多肽之一的任何100%一致性匹配，或者FASTA一致性匹配大于45%的任意100个或者更多氨基酸片段，或者E值小于1×10^{-15}。非早熟禾亚科的其他禾本科植物来源的基因引起乳糜泻风险很小，即使与引起乳糜泻的谷蛋白是同源的，它们导致疾病的可能性也不大。如果蛋白不超过上述的标准，诱导乳糜泻的风险应该很小或者无风险，不需要额外的检测。

4.9 潜在的毒性

少数蛋白被食用时是有毒性的，且大多表现为急性毒性(如蓖麻毒素)[70]。除了用生物信息学方法将任何一个新表达的蛋白比对关键词为“毒素”或者“有毒”的NCBI蛋白数据库以外，安全使用历史的评估也是重要的考虑因素。尽管似乎缺少发表数据来说明如何对于转基因生物潜在毒性进行生物信息学评估，但所有提交到FDA或EPA的转基因产品都必须接受评估[71]。针对监管意见书，我也对一些提交监管审批的潜在转基因作物和新的食品原料进行过生物信息学搜索，在关键词为“毒素”或者“有毒”的限定下BLASTP比对搜索NCBI蛋白质数据库，将重点集中于潜在风险上。通常对于新蛋白的搜索还需要额外的序列比对，在没有关键词的限制条件利用新蛋白进行搜索比对，提供已知有安全使用或者人类安全接触历史的蛋白质与查询蛋白质(转基因蛋白质)序列或者新的食品添加剂的比对。这个过程还需要一个对已发表的科学文献细致的评估，这些文献应该与目的蛋白和搜索序列最接近序列相关。虽然生物信息学家经常声称，序列一致性大于25%的蛋白质具有同源性和相似的功能，但是大部分具有如此低序列一致性的蛋白质，并不一定具有相同的特异性毒性性质或者完全一致的酶学功能。因此，相对其他蛋白质的生物信息学评估结果也需要被评估。这个评估用于决定是否需要任何毒理学测试，如果需要，需确定测试的靶器官，以及哪些测试对于评估风险可能有用。到目前为止没有证据表明在美国被批准的任何导入转基因作物的蛋白对人或其他哺乳动物有毒性。

在美国的监管体系中，如果一个转基因作物中被转入的蛋白质可能对昆虫、细菌、真菌有毒或者具有抗病毒活性，比如植物整合的杀虫苏云金芽胞杆

菌晶体蛋白，这样的蛋白必须通过小鼠的急性毒性试验。OECD 对于急性毒性试验的指导原则(E425，2001)是很多研究遵循的模式。成年小鼠被灌胃的蛋白剂量通常为每千克体质量 1 mg 蛋白量的 1 000 倍，(每千克体质量 1 mg 蛋白量)是人类食物摄取量的期望值。有时候超出剂量不会这么高，但一般也可达到 100 倍的剂量。从剂量给予当天开始，在有对照(给予模拟剂量)的条件下进行 14 d 的动物健康监测。进行体质量、血液样品观测和每日临床观察的数据收集。对动物实施安乐死并进行整体病理学检查，如果需要则进行组织学样品检查确定是否有异常。通常每个处理组的每个性别有 10 只动物。一般会有 2 个剂量的实验处理组以保证任何异常有剂量效应。尽管在转基因组和对照动物组之间，一些测量值可能会有统计学的差异，同一品种老鼠的历史体质量和测量值可以用于对非期望差异的评估。一些对批准的转基因产品进行小鼠急性毒性试验的研究已经发表[72-74]。很少情况下，更长的毒理学研究会被监管机构或者技术上的专家要求，但是额外的毒理学测试对于增加科学上的判断依据意义不大。非常重要的一点是，考虑到不同于许多的有机物或者重金属，摄入的蛋白质不会在动物体内富集，来自蛋白质的毒性效应应该是急性的而非慢性的。

一些国家(如欧盟)要求进行急性小鼠试验，以及亚慢性的大鼠90 d全食物喂养研究，或使用高剂量蛋白质的重复剂量试验。对于 90 d 研究的试验设计，OECD 的指南有详细描述；一些已发表的研究论文也进行过验证，但是没有很好的理由或者充分的证据来证明这样的研究可以鉴定已知的危害[75]。90 d大鼠喂饲研究更多的是一种毒理学和营养学的混合研究。一些监管者和建议者认为 90 d 研究提供了一个评价由于新基因的插入作物基因组而引起“非期望效应”的工具。值得考虑的是，对于宿主(受体)作物我们通常食用了几个世纪，具有安全食用历史，并且通过自然繁种品种和品系的遗传变异导入了很多非期望的遗传改变，但是并没有在食物中引入有害的毒性。

有 2 个研究使用了重组水稻进行大鼠 90 d 喂饲试验检测一些预期值，得到了一些相互矛盾的结果[76-77]。第 1 个试验喂饲了表达来自雪花莲(GNA)凝集素的转基因水稻，作者的结论是该研究未能发现雪花莲凝集素具有潜在毒性。第 2 个实验是表达高水平菜豆凝集素 PHA-E 的转基因水稻，当蛋白以原始状态高浓度喂食时表现出了毒性。我理解的是当人们食用未加工的芸豆或者菜豆时，未煮过的 PHA 具有明显毒性，这和预期是一样的。对雪花莲凝集素的研究结果似乎是阴性的，可能是因为蛋白的表达浓度太低或者在饲料制备过程中被加热了。由于人类可以食用煮熟的芸豆和菜豆，而不是生的豆子，这似乎使得实验结果预测了人类的经验。试验应该设计未加工的和煮熟的 2 组单独的处理，这样似乎更合适。这个测试也不是很灵敏，而且相对于人类的饮食，喂食的剂量非常有限，远远小于毒理学研究中通常使用的 100 倍安全系数。许多毒理学家质疑 90 d 的全食物喂饲研究是否真的有用[9]。而另一些科学家则主张需要更详细、更复杂和更昂贵的研究来充分检测潜在毒性[78]。然而，最近对已发表的针对转基因作物安全性、毒理学以及全谷粒的大鼠喂饲研究，进行一个同行评估对这种评估方法作出了客观的评价，结论是大多数情况下 90 d 喂饲试验是没有必要的，而且结果也是一致性的安全[79]。有趣的是，在动物人道主义协会和动物保护和利用委员会呼吁减少动物实验的同时，一些参与监管或者转基因生物检测的科学家们呼吁开展更多的未证实的动物研究。

4.10　额外的毒理学研究

对于任何新建议的毒性测试和已存在的测试方法有几个问题是需要考虑的。在已有的测试方法中，什么类型的危害可以或已经被确定？每个试验的假阳性和假阴性率是多少？最后，有没有更有效的试验可用？最近许多发表的文献讨论了使用替代性的基于计算机、基于细胞或者基于组织的方法(包括赞成和反对意见)，主要针对的是药物的毒理学评价[80-81]。讨论集中于科学上合理的假说、经过验证的方法和历史参照数据作为基础标准。了解不同模型的局限性和优点对于决定试验是否可用于评估潜在毒性是至关重要的。在大多数国家，包括美国，动物保护和利用委员会有一个基本要求，除非有理由怀疑结果，否则不能在之前没有应用过的特定试验材料上进行特定测试。因此，相同的转基因作物的事件在多个国家重复相同的动物试验，被认为是不道德的。

毒性评价的最终结果应该是相对于类似的非转基因品种，转基因作物不会造成任何额外的显著毒性风险，或者是构成实质性的新风险。如果研发者

遵循标准的评价过程，对许多新转基因产品，FDA和EPA就能够作出结论。不幸的是，欧洲（如EFSA和欧盟委员会）、印度以及中国的一些监管机构，继续提出假设性的新问题，包括潜在的免疫辅佐，导致消费者生育问题或诱发消费者癌症，即使只有极少的例子，膳食蛋白质具有这样的效应。因此这些监管机构不批准这些没有风险证据的产品。美国监管机构强调需要使用经过验证的方法来评估新的蛋白质和转基因产品的安全。他们没有要求（到目前为止）还没有证实对安全评价有帮助的其他研究。不过，如果研发者提供从新检测方法获得的数据，他们也会进行考虑，虽然这样会使审批延迟。

4.11 转基因产品的非预期效应评价

相对于通过突变或生殖等“自然过程”，产生抗除草剂和抗虫性状（这些性状已经过监管审批并广泛应用）所使用的遗传修饰的方法造成的宿主基因组改变是很小的。有趣的是，这种“未知的”自然变化只能通过表型变化来描述，并且这些方法已经被接受，它们提供必要的遗传背景多样性，使得植物可以在病虫害威胁、气候条件及土壤多样性的环境中生存下来。相比之下，转基因生物却可以从插入位点、拷贝数、基因序列和编码产物几个方面来精确地描述其特性。如果引入了一个编码酶的基因，就必须对这个酶的代谢产物进行评估。1948年，在芭芭拉·麦克林托克的研究中描述了在玉米（*Zea mays*）基因组中有相当一部分是由被称为“跳跃基因”的转座子组成和改变的。由于她的这项发现，芭芭拉·麦克林托克获得了1983年的诺贝尔生理学奖[82]。最近的一项研究表明，在玉米的503个遗传上不同的家系中，有大约16%的基因不存在于所有的503个家系[83]，表现出显著的遗传变异。人们今天所食用的面包小麦（*Triticum aestivum*）是由3套相对原始的禾本科物种的染色体（因此是进化上的六倍体）组成的，所以，小麦中的大多数蛋白是由3套相异的基因编码的，有些几乎相同，有些差异显著。除此之外，在有性繁殖过程中的基因组复制可以造成获得或缺失某些基因功能，改变植物在不同环境下的生长能力或对营养物质（或抗营养因子）进行选择。面包小麦和意大利面小麦（硬粒小麦和圆锥小麦亚种，硬粒小麦是进化上的四倍体）都具有很高的营养价值并且在人类食品中有着广泛的应用。但它们可以导致一些人产生乳糜泻，这样的人口占北美和欧洲总人口的1%，遗传上易感个体占总人口的25%，有小部分群体（<0.4%）会产生IgE介导的食物过敏反应。这2种小麦都属于非转基因作物，目前相关部门还没有批准转基因小麦品种。这说明，所有的食物对于一些消费者都存在某些风险，通过遗传变异为每一位消费者生产可以食用的食物是非常有用的。

我们应该退一步想想，为什么我们吃某些食物，如大米、小麦、大豆、玉米和其他食物，却不仅仅只吃谷物。人们选择了某些便于生产的特定食物来源，但更多的是看重其营养价值。一般情况下，营养价值可以从能量、氨基酸组成、脂质含量、碳水化合物、维生素和矿物质几个方面来衡量。很久以前从这些作物初次种植和消费到现在，在没有任何科学方法测量其营养价值的情况下，作物通过育种与栽培已经发生了很大的改变。在过去的100年里，我们已经知道怎样去测量营养物质的组分，并且在很多情况下知道什么样的饮食是“健康”和“营养”的。通常就是食物多样化。尽管我们知道所有的信息，但我们不会详细地测量每一批制成麦片或面包的作物的所有组分，因为这样的花费是巨大的，同时，我们也知道麦片和面包在安全和营养方面都是合格的。我们已经知道了每种主要粮食作物的基本组成成分，并且有农业营养学测试的典型数据以确保这些农业上重要的物种可以做出最佳的饮食。每一种作物的特定组分也已经进行过评估，营养学家也划定了可以接受用作动物饲料的范围。

4.12 关键营养物质和抗营养因子

转基因宿主植物的关键营养物质和抗营养因子都需要进行检测，并与非转基因品种或同等用途的品系进行比较，期望转基因的关键营养物质不超过遗传背景相似的非转基因品种的合理范围[84-85]。在测定许多组分时，使用不同的统计学方法，会得到不同的统计学显著性。统计上的差异不能作为评判转基因作物不安全的标准，应该有一个基于科学的基础理论来评判产品潜在危害性。为了给特定粮食作物的组分性状提供指导，必须找到相同作物的不同品种最近的历史数据或者在多次田间试验的临近小区种植大量的商业化的品种。

动物营养学家知道选择不同的组分进行检测对于油菜、玉米、棉花、马铃薯、大豆和小麦是非常重要的。并不是所有的组分都需要进行检测，因为有些组分的测定对于这些作物的典型用途是无关紧要

的。然而一些转基因生物监管机构和建议者期望研发者会检测转基因生物的每一个可能的组分，并将其与最近缘的品种进行比较。如果有显著性差异，一些人就认为这是由于 DNA 插入造成的并且该食品是不安全的。我们还了解到，具有相同遗传学背景的植物生长在附近或相距 100 英里外时，由于微环境的差异会导致多种组分上的差异。基因型和环境互作可能导致重要农作物的某些组分的表达产生显著性差异，它们之间的复杂性没有根据生物学相关性进行充分的评估，一些科学家正在呼吁增加使用各种组学来进行高精度的测量。幸运的是，即使组分分析被认为是转基因作物安全性评估的重要组成部分，在美国和大多数国家的监管机构都没有因为组分上的微小差异而阻止批准转基因食品或饲料作物，因为很明显，非转基因的产品在组分上具有的显著性差异不会对食品和饲料的安全评价产生实质影响。最近，两个不同的研究团队发现转基因植物与近等基因系之间的成分差别主要是由于回交和常规育种造成的，与基因的插入无关[86-87]。了解变异的来源是一个需要考虑的重要方面，因为一些作者认为使用蛋白质组学分析转基因植物，内源性致敏原水平的潜在差异也许是由于插入造成的，因此要求额外的测试[88]。

因此，我们需要进一步的定义需要检测的重要组成成分和以及这些组分变异具有生物学相关性的指导。为了给组分提供一些参考，在生物技术产业的支持下，国际生命科学学会（ILSI）建成了作物组分资料库[89]，这个数据库包含了玉米、棉花和大豆种子组分数据（https://www.cropcomposition.org/query/index.html）。版本 5 的数据量是之前数据库的 7 倍，之前这个数据库只包含了 1995－2005 年的几个特定栽培国家的数据。在日本建立的一个数据库（http://afdb.dc.affrc.go.jp/afdb/index_e.asp）中，可以查到水稻和大豆的信息。在日本数据库中，可以获得这 2 种作物部分品种和有限年限的数据[90]。这些数据库提供了相关的方法和不同物种组分范围的一些信息。有趣的是，饲料行业对于商品作物的组分变化最为敏感，因为饲料质量轻微的变化对主要动物生产商来说可能意味着盈利或亏损。比如具有 4 000 多家禽养殖场生产鸡肉的泰森（美国）公司和美国的第二大家禽生产商柏杜农场（美国），会检测饲料中不同作物的重要营养组分含量。为了给禽类的生长和安全制定最佳的饲料配方，他们对每次交货的大量商品作物进行随机抽样并做大致测量分析，测量总蛋白、脂类、碳水化合物、灰分、纤维，常常也会测量氨基酸组成和作物特定的维生素、脂肪酸和矿物质。他们同样测量作物特定的毒性和抗营养因子。家禽业对营养品质的变化是最敏感的。除此之外，由于玉米是最易被污染的作物，每一批玉米粒或干酒糟都要检查霉菌毒素水平。一项转基因作物复合性状在肉仔鸡中的研究，描述了饲料原料组分的评估并提供了真实的数据，这可能和泰森或柏杜的分析类似[91]。优化鸡饲料的主要组分和方法是在饲料准备的过程中进行评估的，并不包含代谢、RNA 转录或蛋白组学检测。相反，他们关注的是那些有助于提高小鸡生长率的组分（在这些研究中，从孵化开始的 42 d 后体质量增长了大约 35 倍）。饲料效率和体质量增加与营养特性是高度相关的，这种相关性比任何其他动物都要多。商业化生产的饲养场规模和动物数量通常是非常巨大的。给鸡喂食的是脱脂大豆粉，因为大豆中的脂肪酸和脂质组分对于鸡饲料来说不像哺乳动物如奶牛那样重要的。大多数的奶牛场、牛肉、猪肉、山羊和绵羊农场除了检测玉米中的霉菌毒素，不会监控每天运来的饲料，只是在一年当中偶尔取样，如果主要组分的营养价值发生变化就重新制定饲料配方。在美国，提供给监管机构的研究中，将新的转基因家系和近缘非转基因家系及 3 到 5 个其他商业化非转基因家系进行比较，将这些不同的品种或家系种植在多个地理位置以提供多样化的生长环境，对品种中近似和特有的作物组分进行检测和对比。有些国家，一般的转基因生物研发者必须进行多年多个地理位置种植重复试验，向监管机构提供转基因作物与非转基因作物具体成分的统计分析结果。但这与安全性的相关性通常不明确。

除了营养物质，特定的作物也有其特定的抗营养因子需要检测，包括凝集素、胰蛋白酶抑制剂、毒素如龙葵素和高致敏性作物（如大豆）的致敏原等。目前，多数抗营养因子已经有了普遍可接受的范围（如龙葵素的可接受范围是每千克鲜质量 200 mg，Friedman，2006），但还没有致敏原可接受的限制范围[92]。

4.13　检测内源致敏原水平的潜在变化

如果基因受体（宿主）是常见的食物过敏源，美国要求和欧盟推荐考虑转基因插入是否增加内源性致敏原的表达或积累。监管机构认识到，来自不同

过敏源的食物过敏风险是不同的。加工的食品要在标签上真实地进行标注以降低致敏风险。美国和欧盟的食品标签法规要求,主要过敏源来源的所有成分必须标明。这份名单包括了美国8种常见的致敏食物:鸡蛋、牛奶、花生、多种坚果、甲壳贝类、鱼类、大豆和小麦(http://www.fda.gov/food/resourcesforyou/consumers/ucm079311.htm)。此外,在美国除非麦麸含量小于20 μg/g,含有小麦、大麦和黑麦麦麸成分的食品都必须在标签上标明。在欧盟,除了这8种食品还添加了6种食品到清单中:含麸质的谷物(小麦、黑麦、大麦、燕麦、斯佩耳特小麦、卡姆小麦以及这些谷物的杂交种),芹菜(根)、芥末籽、芝麻、扇羽豆和软体动物以及二氧化硫,其相对未分离的成分必须标明其来源(如小麦、蛋、牛奶)。在美国和欧盟,除非加工的成分是免于标识的(如正己烷炼制的大豆油),通常引起过敏的食物来源成分也必须予以标明。小麦来源的淀粉需要标示为小麦源淀粉,而来自玉米、大米或木薯的淀粉则可以简单地标注为加工淀粉,无需标明来源。因此,在美国和欧盟,研发人员必须对转基因花生、大豆和小麦的内源性致敏原潜在变化进行评估。由于菜豆、玉米、水稻不是常见的过敏源,所以无需对它们进行内源性致敏原潜在变化评估。用于评估的方法一般与诊断致敏原产物的致敏原测量方法一致[93-94]。药用级的致敏原抽提物期望显示出与免疫印记相似的定性结合和总IgE结合的变异(50%~150%的抽提物标准平均血清IgE结合)[93,95]。IgE结合使用混合的过敏者的血清进行以比较一批致敏原提取物与上一批提取物。利用SDS-PAGE分离大豆提取物蛋白并与3位独立的大豆过敏者的血清进行Western杂交,对首个耐除草剂大豆(孟山都40-3-2事件)进行IgE结合试验[96]。Sten等进行了更广泛非监管要求的体外IgE结合研究[97],利用10位大豆过敏试验者血清与转基因事件(40-3-2)来源的10个品种和8种遗传背景相似的非转基因品种进行比较。他们利用RAST抑制和噬碱性粒细胞释放组胺,发现虽然个体之间与品系之间明显不同,但转基因大豆和非转基因大豆之间没有显著差异。我的实验室也选取3个不同公司来源的5个转基因大豆事件进行血清IgE结合研究。我们用到的研究方法包括直接IgE结合实验,混合大豆过敏血清的ELISA抑制或独立血清的直接ELISA,除了一个或多个非转基因家系之间存在显著性差异,没有发现任何显著性差异[92,94]。在某些非转基因大豆间被发现存在某些差异,因为在IgE定性免疫印迹实验中缺少或者多了1条IgE结合的带。由于EFSA[51]和欧盟[52]的规定,除了这些评估致敏原丰度潜在变化的标准方法以外,还需要利用2D免疫印迹的方法分离单独个体的血清蛋白,并将每个转基因的与非转基因蛋白家系相比较。一些个体的血清IgE结合位点存在不同之处,但在转基因家系中没有表现出差异[92,94]。显然,在类似的研究中,过敏个体之间的差异会对结果产生影响。除非多个大型过敏中心参与,在一项研究中寻找好几个(10个?)特异性的过敏者是不切实际的。不同过敏者蛋白质和同工异型体与IgE的结合总是存在一些不确定性。然而,对于EFSA建议使用蛋白质组学(LC-MSMS)来评价大豆和其他常见致敏粮食作物的各个"致敏原"的丰度不像血清测试一样有效,因为EFSA希望使用的"致敏原"列表[例如,在OECD的大豆组分列表中的致敏蛋白,包括致敏性证据很少或没有证据(甘氨酸m1、甘氨酸m2、甘氨酸m3(抑丝蛋白)、P34甘氨酸m Bd 30K、未知的天冬酰胺残基连接的糖蛋白、凝集素、脂肪氧化酶、Kunitze胰蛋白酶抑制剂、未知的39和50 ku的蛋白质和P22-25]。已经确定的大豆中重要的致敏原有甘氨酸m5(β-伴大豆球蛋白α-,α'-,和β-)、甘氨酸m6(5-大豆球蛋白)和可能的甘氨酸m4,也被称为SAM22。由于对选择的蛋白没有进行风险分级,且事实上有些致敏源没有发表的证据或某些情况下蛋白序列没有确定,所以EFSA的建议不是基于风险证据的。另外,LC-MSMS不能100%覆盖很多蛋白,因此也不太可能鉴别出共同异型体,这些蛋白中的一些可能不与IgE结合。血清IgE结合实验至少可以应用过敏个体来源的血清比较转基因作物和非转基因作物品种的生物学测量(IgE结合)结果。

但考虑这些对内源性致敏原的测量与安全性是否有相关性是非常重要的。如果有差异,是否会导致过敏的风险增加?对大豆过敏的人应该避免食用任何大豆。对大豆不过敏的人则想吃多少就可以吃多少。在加工食品中,不同产品的总大豆蛋白含量差异非常大,食品公司不会选择太多特殊的大豆品种。相反,他们会大批量购进,并在料仓、运输、制粉和加工、食品制造过程中将大豆混合。

有一个很重要的问题没有任何科学研究或监管机构可以回答。什么程度的改变会导致内源性致敏

原的累积，从而对敏感的、处于高风险、对特殊食物过敏人群的健康产生负面影响。一个可能有用的评估是，经过很好训练的临床过敏专科医师应用间隔性剂量提高做双盲的、有安慰剂作对照的食物测试(DBPCFC)。有一些发表的文献，描述了用已建立剂量阈值的各种致敏原测试高风险患者的方法。Crevel 等人发表的一篇研究综述[98]中报道了对花生过敏试验者增加 3 到 10 倍的花生测试剂量的实验方法。在 EuroPrevall 的 DBPCFC 研究中设计的实验，首先使用 3 μg 过敏源蛋白和增加 10 倍至 30 mg 蛋白，随后当用量超过 30 mg 后只增加 3 倍的用量，因为超过 30 mg 的用量后感觉严重过敏反应的风险会增加[99]。因此，在实验设计时用量至少要成 3 倍地增加。如果世界级的临床过敏专科医师认为使用 3 倍到 10 倍剂量没有问题的话，使用比这个水平低的剂量就没有问题。

4.14　对潜在新的非预期蛋白的评价

在描述研究每一个转基因事件时，都需要对插入的 DNA 进行分析，以确认插入序列以及侧翼 DNA 序列。通常除了插入序列还要鉴定数百到一千不等的碱基数。分析插入的序列是为了确保蛋白被正确表达。如果发生非预期的改变，那就应该对新蛋白的功能进行评估并利用生物信息学方法对新蛋白质的过敏性和毒性风险进行评价。侧翼 DNA 被评价以确定是否可能有新的融合蛋白在植物中表达。DNA 序列中所有的 6 个阅读框都将利用计算机算法进行评估以识别潜在的开放阅读框。一些监管者只要求符合从起始密码子(甲硫氨酸)到终止密码子确定的潜在开放阅读框(ORF)，而另外一些监管者则要求所有假设的即从终止密码到终止密码的开放阅读框。随后利用生物信息学对这些潜在的开放阅读框进行评估分析，搜索匹配的致敏原和毒素蛋白。最关键的部分在于融合位点。插入片段两端的植物 DNA 是已经存在的，如果它编码致敏原或毒素，则是已有的内源性风险。安全性评价主要关注新的潜在危害和风险。如果与致敏原和毒素匹配，则需进一步分析评价是否有以及哪个组织从该 DNA 区域转录了 RNA。如果存在这种特定的 RNA，那么需要使用 LC-MSMS 或由该开放阅读框编码的合成肽产生抗体来检测是否翻译出产物(蛋白)。如果蛋白质水平可以忽略不计的，则风险很小。

一些监管者要求更长的侧翼序列，直到清楚地知道转基因没有打断内源植物基因的编码序列或间隔序列(内含子)。通过地域重复的田间试验来评价植物的农艺性状可以帮助识别转基因与非转基因品种之间的各种生物学显著差异。这种类型的评估是针对植物性状的，而不是安全性。美国的监管机构非常注重收集食品和饲料类产品安全性的相关信息，因此转基因的研发者和相关的种子公司必须向农民们提供数据让他们相信转基因作物在产量和总组分上有足够的产出，否则农民们不会购买他们的种子。

4.15　对非预期效应评价的结论

组分分析的结论通常是指特定作物的总营养物质和抗营养因子是否大体上和非转基因作物实质等同。这些分析通常是通过转基因和非转基因品种在真实的田间条件下进行不同地域重复的田间试验。当然，在测量不同地区众多样本的各种组分时往往会导致一些统计上的显著差异。大部分的变化是由于整个植物基因组的实际遗传差异，回交及各种育种方法对转基因插入没有影响[82-83]。此外，最近的研究发现 DNA 甲基化模式可以遗传并且改变基因的表达而不引起任何 DNA 的变化，这个发现帮我们认识到我们不能期望控制或者了解 DNA 序列信息上每一个可以测量的差异[100]。更重要的是，我们知道并不是每一个可测量的差异都会对消费者构成风险，事实上只有很小的一部分会。人类在几百到几千年前选择并改良了大多数驯化的作物。我们知道遗传多样性使得可以在各种环境条件下都能种植同一物种，来生产食物和饲料。

在美国，对转基因事件和遗传背景相近的作物(近等系或亲本品种)以及同一田间试验的商业种植品种或者实际生产采集的样品的最近记录数据等来源的不同测量数据进行比较。如果转基因作物的测量结果落入偏差范围基准之内，那么这个转基因作物和遗传背景相近的品种之间的差异就被认为是可接受的。转基因作物因此也被认为在实质上等同于这一作物的其他品种。同样的推论也适用于通过饲喂实验测量动物反应所获得的数据，如一些监管者要求的 90 d 老鼠喂养试验；42 d 肉鸡研究或者大型动物喂饲试验在许多国家是行业接受的研究，但一些监管者也对其有要求(如印度)。

4.16　转基因生物审批现状

有多少转基因作物已经得到研发并且获得监管部门的批准允许作为食品和饲料进行种植？目前为

止仍很难找到准确的信息。环境风险评估中心(CERA)转基因作物数据库 www.cera-gmc.org/GmCropDatabase 中列举了总共 153 个作物事件,但并不是所有的这些都是通过转基因技术完成的,其中有一部分是通过突变或者传统育种的手段研发的。此外,并不是所有作物在任何地方都被批准种植,而且其中一些被批准了但没有被使用。国际农业生物技术应用服务组织(ISAAA)还建立了一个转基因作物数据库(www.isaaa.org/gmapprovaldatabase),其中列出了 353 个事件。通过快速查询,ISAAA 似乎有一些没有被 CERA 列出的作物类型,包括豆类(菜豆)、茄子、杨树、甘蔗和胡椒,这些还没有提交给美国或者加拿大的监管机构。很可能每个这样的数据库会遗漏一些转基因事件,但不太可能遗漏目前全球交易的转基因作物。另外美国的 3 个监管机构都有各自独立的显示他们针对个别转基因事件动态的数据库。USDA 的网址是:http://www.aphis.usda.gov/biotechnology/petitions_table_pending.shtml。FDA 的网址是:http://www.accessdata.fda.gov/scripts/fdcc/?set=Biocon。EPA 的网址是:http://www.epa.gov/oppbppd1/biopesticides/pips/pip_list.htm。

尽管许多不同特性的转基因事件被批准使用,但是大多数的事件存在于少数大宗商品作物中比如油菜、棉花、玉米和大豆等。通过在美国测量种植转基因作物面积的百分比可以看出转基因作物的采用率增长非常迅速,从 1994 年的零增长到了 2014 年的大豆和玉米超过 90%。在美国棉花产量中很大一部分来自转基因作物,而在印度 95%的棉花以及中国 90%的棉花是转基因棉花。现在许多来自不同研发者的转基因作物都有相似的功能,比如对除草剂的耐受性和特定的抗虫性。与此同时,许多以前批准的转基因作物(1994 年之前)已经从市场上消失了。一些产品被撤销是由于受到了消费者和公司方面的压力,比如孟山都公司研发的抗病毒、抗马铃薯甲虫的转基因马铃薯。由于在马铃薯市场占据主导地位的是炸薯条和快餐店,他们对于消费者的偏好非常敏感。这些产品可以大幅度地减少马铃薯杀虫剂的使用,由于来自快餐行业的压力于 2002 年退出市场。孟山都公司向美国和加拿大提交了耐除草剂小麦的申请,但在未获审批前就撤销了。这是因为来自加拿大小麦协会的压力,他们担心这些小麦出口亚洲会有贸易障碍。延熟番茄没有被商业化应用就退出了(有 4 个公司包括捷利康和孟山都公司的获批转基因事件),因为鲜食的质量没有非转基因的品种好。陶氏益农公司开发的抗病毒瓠瓜仍然在市场上,但是已转让给圣尼斯公司。抗病毒的木瓜由康奈尔大学的研究者研发,目前批准在美国使用,因为夏威夷的木瓜树受环斑病毒危害严重。转基因载体可以阻止病毒复制,引入这个性状拯救了夏威夷的木瓜产业。

4.17 小 结

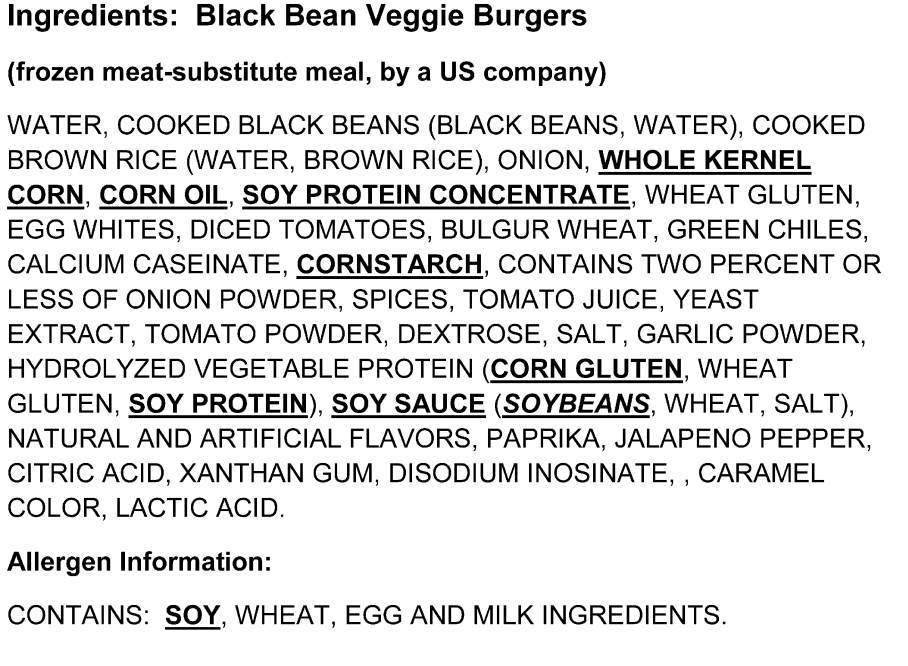
一些专家预测会有严峻的全球粮食危机,而另一些专家认为是由部分地区的干旱、疾病、人为政策或者经济问题而引发持续性的区域化危机[101-102]。生物技术及现在和将来的转基因作物可以作为改善全球可持续农业的部分解决方案。但其进展受到过于专注且有资金支持的少数非政府组织和知名人士的压制,虽然这些知名人士并没有很好地理解农业、粮食生产、饥饿和成本问题,却依然激起了民众的疑虑。在这场争论中我们能找到共同点吗?少数食品生产、农业和人类健康的学生可能否认,虽然在过去的一个世纪生产效率显著增加,但世界日益增长的全球人口将超过长期维持粮食生产的能力[103]。然而,目前我们提高生产的能力是通过使用开采的矿质作为肥料,在耕作中增加化石燃料的使用,用机器代替人力劳动,和在劳动密集的区域使用牲畜而实现的。那我们可以保持目前的扩张速度吗?通过美国农业系统的经验,可以为中国及其他亚洲国家利用转基因作物的益处并对建立新开发产品的食品安全标准,提供有用的参考实例。

考虑到美国在安全评价过程及转基因作物法规方面的经验,有必要看一下全球粮食供应的性质、各种食品安全监管机构的担忧以及各种粮食作物的悠久种植历史。没有一个国家是自给自足的,任何一个国家大多数食用的食物都起源于其他国家,或者在一定程度上依赖其他国家的输入。我们今天食用的小麦、大米、马铃薯、番茄、辣椒及各种豆类和特定的动物,都是由自然出现的原始生物从不同的地理区域进化而来,而不是像现在这样生产出来的,并通过数百年甚至数千年的育种过程筛选和改良。这些食物是基于食物的实用性(营养和抗营养)、易于生产和食用安全等特性而选择出来的。当然也存在人们对食物过敏及乳糜泻的风险。对某些人也有烹饪或储存不当导致没有抑制微生物和腐败和灭活抗营养因子的风险。而新蛋白质的主要潜在风险通过当

前法典的评价框架是相对容易避免的。

还有一些不确定性使美国的监管机构仍然不能理解。首先，如果蛋白质对胃蛋白酶测试是稳定的，那这个蛋白质可能使人们对其变得敏感而成为致敏原。低丰度的稳定蛋白质有低风险，但应该找到一个可以接受的标准。我们还需要继续努力以一个更好的方式来预测致敏原。当前计算机程序预测建议的抗原性远不完美，并且会有过度预测的风险。到目前为止，动物模型没能很好地提供有效精准的预测。基于人体抗原呈递细胞为基础的细胞检测，T 细胞和 B 细胞没有显示出精准的预测。在当前法典指南和美国的评价过程没有显示安全结果的一些困难蛋白，需要额外的研究。但现在转基因产品很容易通过生物信息学避免过敏、乳糜泻和毒性的问题。在一些情况下，血清 IgE 检测或者简单、预测性的毒性测试也是必要的。

食品标识是一个全世界的主要问题。一些国家像中国一样要求含有转基因成分的食物进行标识。在美国一些州，标识制度已经通过了法律（可能在不久的将来生效），还有一些州将在 2014 年 11 月就标识问题进行投票。食品生产经济上和实际上的障碍使得标识制度难以执行。作物种植和交易跨越州界和国界，食品公司的产品通常销往 50 个州甚至出口。有个别的材料可能含有转基因成分，但是不同批次的情况不同。例如，图 2 显示了在美国生产的黑色素食豆类汉堡的标识成分。来自大豆、玉米、油菜、棉花的组分都可能是转基因的。CERA 转基因作物数据库（http://cera-gmc.org/index.php/GM-CropDatabase）列出 12 种被批准的转基因大豆，代表了 8 种转基因蛋白，57 种被批准的玉米代表至少 15 种不同的蛋白。如果这些法律通过，并且商品、原料和最终食品的供应商不想被贴上“转基因食品”标签的话，他们必须控制和检测所有的这些材料。这样将增加费用，并且对安全性没有益处。对于食品，那些已经杂乱的标签信息，使得关键的安全信息如致敏原成分都丢失了。



Ingredients: Black Bean Veggie Burgers

(frozen meat-substitute meal, by a US company)

WATER, COOKED BLACK BEANS (BLACK BEANS, WATER), COOKED BROWN RICE (WATER, BROWN RICE), ONION, **WHOLE KERNEL CORN**, **CORN OIL**, **SOY PROTEIN CONCENTRATE**, WHEAT GLUTEN, EGG WHITES, DICED TOMATOES, BULGUR WHEAT, GREEN CHILES, CALCIUM CASEINATE, **CORNSTARCH**, CONTAINS TWO PERCENT OR LESS OF ONION POWDER, SPICES, TOMATO JUICE, YEAST EXTRACT, TOMATO POWDER, DEXTROSE, SALT, GARLIC POWDER, HYDROLYZED VEGETABLE PROTEIN (**CORN GLUTEN**, WHEAT GLUTEN, **SOY PROTEIN**), **SOY SAUCE** (***SOYBEANS***, WHEAT, SALT), NATURAL AND ARTIFICIAL FLAVORS, PAPRIKA, JALAPENO PEPPER, CITRIC ACID, XANTHAN GUM, DISODIUM INOSINATE, , CARAMEL COLOR, LACTIC ACID.

Allergen Information:

CONTAINS: **SOY**, WHEAT, EGG AND MILK INGREDIENTS.

Ingredients that may contain a currently approved GMO are listed in bold and underlined. Those ingredients may be subject to GMO labeling laws if mandatory labeling laws are passed. The Allergen information is a safety label as it shows major ellergenic ingredients that have to be avoided by some consumers with specific food allergies so they would know to avoid this product for safety reasons if they are allergic to soybean, wheat, eggs or mili.

图 2　2014 年美国生产的商业黑豆汉堡的成分标签

Fig. 2　Ingredient label of a commercial black bean burger produced in the US in 2014

人类消费的食物在组成和营养品质上极其多样化,这本来就有危险。我们是杂食动物,我们的祖先适应了不同的气候和条件,遍及大陆各处,从迁徙的狩猎者到游牧民族,最后成为相对稳定的农耕者[104-106]。从人类的可以合作和接受由于帮助他人生存(而不仅仅是保护自己的家庭)而增加成本的能力来看,这种适应似乎已经变为可能。但这种适应有时对直系亲属没有益处,但对社会是有益的[106]。在后工业时代,人类个体已经变得高度机动。然而,由于种种原因,在每个社会维持人口所需的基本食品生产基础设施变化缓慢,这些原因中包括设备投资巨大、商品及食品加工设备的复杂性、用于生产的植物及动物基因资源的限制。但是适应发生了,生产效率也增加了,尤其是在20世纪。随着人口远离粮食作物生产而集中在城市,可用耕地增加了[107]。世界人口现在估计超过72.5亿,总生物量超过了所有其他的陆地脊椎动物生物量的总和,我们需要认真考虑如何改善食物和饲料生产。人类花了数百或数千年才学会如何管理和接受许多食品生产的新方法。在过去的100年里,食品生产已经明显地转变为工业化的方式,来适应食品的需求。一些人试图阻止新技术,限制将新改良引入粮食作物的工具,因为他们声称这将生产出不安全的食品。但是当我寻找转基因危害的证据时,发现并不存在。

现在我们生产及消费的植物食品没有一个是完全自然的。虽然从遗传学上讲它们与一些本土植物非常相似,但谷物(小麦、大麦、黑麦、水稻、玉米、高粱)已经被选育了数百年。番茄、马铃薯、茄子、辣椒的许多品种经过不同的烹饪过程后食用是安全的。但它们与其他茄科包括有毒的茄属植物如不能食用的烟草及矮牵牛关系密切。可食用的茄属植物同属一种的野生近缘种中产生足够水平的配糖生物碱(茄碱、番茄苷及其他)和凝集素,如果食用则会对人及家畜相当有害。我们仅能食用这些作物中的当前这些品种,因为我们的祖先经过育种和筛选,这些植物的可食用部分含有低水平毒素和抗营养因子。他们完成这些工作,并没有我们今天使用的复杂科学测试和仪器来检测引起伤害的特定物质,也没有标准化的动物喂饲试验。虽然我们是杂食动物,可以食用不同的植物和动物,但我们必须知道我们能够食用的限制是什么。我们今天吃的马铃薯是安全的,但我们已经了解到一些野生近缘种能产生足够高浓度的茄碱、番茄苷和其他配糖生物碱,足以引起危害甚至死亡。

超越历史的角度来看,同样重要的是要记住,我们生活在一个频繁且无意识影响错误信息传播急剧增加的时代。许多人声称各种食物中真实或者潜在的危害是几个世纪或者几十年前根本没有引起注意的,但引发恐惧的往往是假设的危险。然而即时通讯和互联网使时间变短。16世纪,当欧洲探险家把番茄和马铃薯从南美带入意大利和英国,他们在南美被引入并且种植和安全食用了1 000多年。但是欧洲人没有足够的知识种植和安全食用这些植物。一些人由于不正确的食物烹饪,或者食用植物的绿色部分而生病,生病之后,人们认为整个植物包括果实和块茎都是有毒的。南美洲的原住民知道避免食用绿色的植物材料。在欧洲的这些极少数的食物中毒事件导致了人们广泛的恐惧,拒绝现在这些主粮进入人们的餐桌。现在关于转基因的虚假报道俯拾皆是,并产生持久的影响。最近奥兹博士、杰弗里·史密斯、奥普拉·温弗瑞和崔永元声称转基因作物不安全或未经检验,导致消费者对声称转基因作物安全的生物技术公司和政府产生怀疑。然而这些媒体名人并没有阅读文献或者美国监管部门为确保像MON810(抗欧洲玉米螟)这样的产品是安全的而开展的安全性研究。当那些"可信的"知名人士告诉消费者政府是多么的腐败,像孟山都这样的大型生物公司并没有做足够的测试和安全评价工作时,我们该怎样向消费者陈述事实呢?

5 结 论

美国监管机构评价转基因作物安全性涉及3个联邦机构:USDA、FDA和EPA。这个评价程序始于20世纪80年代末和20世纪90年代初,由学者、企业科学家、政府监管的科学家及政策制定者磋商而来。在20世纪末到2003年,通过与食品法典委员会关于转基因作物安全评价指南相结合而得到完善。对转基因作物来源食物中潜在的过敏性风险的评价须使用科学上可以接受的方法进行。这对于鉴定可能出现食物过敏风险的蛋白很有效。这个蛋白可能是一个经转移的已知的致敏原或是一个交叉反应的蛋白。但仍然有一点不确定:如何预测一个没有明显风险的新蛋白是否会导致新的过敏呢?不过,如果这个蛋白在胃蛋白酶体外测试中会被迅速降解,或者该蛋白在食品中的含量比较低,这种风险是比较低的。与非转基因品种相比,转基因植物显

著提高内源致敏原表达水平的可能性是相当低的，但对于原本就要避免食用寄主植物的那些消费者存在风险。因此，即使内源致敏原含量增加了，其风险也没有实质性地增加。基于非转基因生物确立的食物毒性评价原则可用于转基因生物。少数蛋白是有毒的，将转基因的蛋白序列与已知有毒蛋白的序列比对，同时评价基因来源及该蛋白的作用机制，将鉴定出高风险的蛋白。美国已经评价并批准了大约 100 种转基因新事件或新品种的商业化。目前还没有关于人或动物因食用转基因植物可食用部分而中毒的记录。然而监管过程花费昂贵并且消耗时间。粮食作物的交易是国际化的，但遗憾的是，目前没有一个标准的、各个国家都可以接受的安全评价程序来避免重复研究。

致谢　感谢美国科学院和中国科学院，为我参加在武汉召开的讨论安全评价的联席会议提供资金支持。我的实验室也很幸运得到 EPA 关于转基因作物致敏性评价改进的 3 个研究的经费支持。如同盖茨基金会通过丹弗斯植物科学中心，美国农业部 FAS Borlaug 计划资助我们就转基因作物致敏性评价培训了大批国际学者。我不但有幸能够帮助非洲、澳大利亚、印度和美国一些科学家，评价大量重要转基因产品潜在的致敏性和毒性，而且受到 8 个农业生物公司提供资金来维护 AllergenOnline 数据库，并对转基因大豆内源性致敏原的潜在变化进行研究。最后，能和一群有天赋且工作努力的研究生、技术人员以及过敏方面的专家工作，并探讨数据库和其他致敏原的问题是非常美妙的。

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Biosafety：Evaluation and regulation of genetically modified（GM）crops in the United States

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Abstract　This review of the safety assessment of genetically modified（GM）crops is focused primarily on the process and progress in the United States（US）. It reviews the development of the safety evaluation process from the Asilomar conference in 1975 considering issues relevant to recombinant DNA technology，to discussions between the US government，academic and industrial scientists between 1984 and 1994 when the first GM crops were being field tested and evaluated commercial release for food and feed production. International guidelines were also reviewed for consistency with the US system. The overall process includes consideration of information relating to history of safe or unsafe human and exposure to the gene source and expressed proteins. The primary considerations of safety for dietary proteins are whether or not some consumers are sensitized and have IgE antibodies against the protein encoded by the transgene or whether the transgene represents a risk of eliciting celiac disease. The process considers potential toxic effects of expressed proteins as well as potential impacts on human and animal nutrition. The process in the US is consistent with Codex Alimentarius recommendations. It follows a science based process based on justifiable hypotheses. To date there is no evidence that GM crops approved in the US have harmed human or animal consumers. The evaluation takes into account genetic and environmental variation in products produced by plant varieties and is intended to maintain the standard that foods developed from GM plants are intended to be as safe as non-GM genetically similar varieties.

Key words　genetically modified crops；food safety assessment；allergenicity；toxicity

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Biosafety: evaluation and regulation of genetically modified (GM) crops in the United States

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Keywords genetically modified crops; food safety assessment; allergenicity; toxicity

1 Introduction

The safety of foods produced from genetically modified (GM) organisms, GMOs or GM crops is mandated by most countries including the US, China and countries who are members of the Codex Alimentarius Commission, an international food standards program within the World Health Organization and the Food and Agricultural Organization of the United Nations (www.codexalimentarius.org). The Codex includes 185 member countries plus the European Union (EU) and has 224 official observers (non-member countries plus non-governmental organizations (NGOs) and outlines guidelines for many important questions regarding food safety and international trade.

The overall process of evaluating the safety of foods produced from GMOs has been described in only a few documents. The primary food safety guidance for developers and regulators of GMOs is the combined documents of the Codex Alimentarius Commission, Second edition, "Foods Derived from Modern Biotechnology"[1]. The principles are outlined in the first chapter (CAC/GL 44-2003) and include definitions of organisms derived from "Modern biotechnology", or genetic modification and the risk assessment process that is intended to identify any new hazard or nutritional or safety concern presented by the new GM organism as well as risk management procedures if appropriate. Three major sections follow that are intended to define processes to evaluate the safety and nutritional properties of GM plants, GM animals and GM microbes. The assessment strategies are quite similar for all three. The food allergy assessment is outlined as a separate annex at the end of the chapters. Evaluation of potential risks of toxicity, celiac disease and nutritional equivalence are discussed in the section on substantial equivalence toward the front of each of the documents. The Codex[1] document is a guideline for individual countries that have to develop their own regulations. The intent of Codex is the signatory parties should develop regulations that are consistent with these guidelines unless differences are scientifically justified. Since the documents were written from 2001-2003, there have been some advances in the science

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and those will be discussed along with commonalities. Newer evaluation steps will be discussed here. This paper will focus primarily on the assessment as performed in the US. However a review of some of the concerns and criticisms of people and organizations who opposed GMOs will also be discussed as it is important to understand whether there are legitimate safety issues that are not being addressed by regulatory bodies and developers.

The safety of foods derived from GMOs is a focus of some NGOs including Greenpeace, Friends of the Earth, Union of Concerned Scientists as well as personalities on popular television and the internet in the United States (US), European Union (EU) and China. We must recognize that it is natural for consumers to be concerned about food safety, especially regarding new foods or technologies that many individuals do not fully understand. Most consumers do not understand the scientific basis of allergens and allergies, toxins, nutrients and anti-nutrients in foods. Most consumers also do not understand the importance of genetic diversity in food crops to enable production in diverse environmental conditions[2]. Many consumers believe that all soybeans are identical unless they have been modified by genetic engineering. Scientists in other disciplines also do not understand the tremendous variation and complexity in the normal composition of proteins, oils, carbohydrates and metabolites of all food crops. A major focus of plant breeders is to introduce variation and if we consider the principles of genetic engineering and look closely at the changes, it is clear biotechnology introduces minimal uncertainty compared to the natural or induced mutations that breeders have relied upon to develop useful new varieties[2]. Can we explain realistic risks to consumers and also explain how the current safety assessment process minimizes risk?

An important concern that is often voiced by the opponents of GM crops and of the pharmaceutical industry is that the companies developing the products are the ones who test for safety. That concern seems reasonable, but needs to be considered in the context the entire legal framework, governmental and economic structure of each country. Scientists in the government of the US and many other countries do not perform safety testing for most products. It is worth noting that no government in the world has enough scientists with the right expertise or enough money to perform the appropriate safety tests for all potential products in a reasonable amount of time. Development of many important products would stop if developers had to wait for safety testing by their governments. The regulatory systems established by most, including the US is to have a number of quality scientists in the regulatory departments who can review safety data critically and make decisions based on protocols and guidelines. Governments like the US have legal mechanisms for consultations with academic experts to assist in the evaluations. The regulators also should have the ability to efficiently communicate with developers to ask for additional data or tell them what additional tests or questions must be answered to gain approvals.

I am most familiar with Monsanto as a major GM crop developer. They have approximately 600 college educated (BS, MS and PhD) individuals working in the regulatory division of the company. These scientists plan and conduct safety and environmental studies, archive and characterize test substances (plants, seeds, DNA constructs and proteins), perform tests, analyze data and write reports for submission to regulatory agencies. They have to grow plants in different environments and sometimes multiple countries in order to perform field and environmental tests. The regulatory process is extremely complex even for one product and development and regulatory approvals for each product often takes ten to fourteen years. Companies like Monsanto also have separate quality assurance units (QAU) that report to a different management team from the development and sales divisions. The QAU reviews protocols prior to study conduct and audit data and reports before they are submitted to regulators to ensure study adequacy and accuracy. Their scientists evaluate mountains of data and develop the dossiers that are submitted to multiple governments before a product is allowed to be grown commercially. Most will gain approvals in major trading countries (Australia, Canada, China, Japan, Korea, the US and Taiwan) before releasing seeds of a new GM product to farmers. International trade of commodities and foods and feed will only work if the developer is managing materials and data as they have the capacity and incentive to ensure timely and coordinated processes. In some cases regulatory studies are performed by contract laboratories, especially toxicology studies as there are regulations that are very strict that require specific tests to be performed following “Good Laboratory Practices” (GLP) as defined by the Environmental Protection Agency (EPA) of the US government. Few developers have the infrastructure to meet all GLP requirements. The toxicology contract companies specialize in meeting regulatory demands. Those studies can be audited by

EPA. There are strict rules about record keeping, ethics and integrity of data. It might be important to consider that if the government were to perform those studies, who would audit them and hold them accountable?

Some studies are performed by academic laboratories because neither the developer nor any contract GLP laboratory has the right expertise to perform the study. My laboratory at the University of Nebraska has performed a number of allergenicity studies (human serum IgE binding and bioinformatics studies) for biotech companies and non-profit agricultural organizations as well as food companies developing novel ingredients. We have collaborations with clinicians who arrange samples from specifically allergic patients who are willing to contribute serum samples to evaluate product safety. We develop protocols, perform the studies, evaluate data, write reports and maintain records related to the studies. We do those studies under contract with the developers under ethical standards managed by the University of Nebraska as well as ethical standards of any collaborator's institution.

I have been involved in designing, performing or reviewing safety studies on allergenicity, toxicology and nutritional qualities and performance of GM crops and novel food ingredients for 17 years. I was at the Codex Alimentarius Task Force Working Group meeting that was held in Vancouver Canada in 2001 that developed the allergenicity guideline[1]. I have been involved in safety studies and reviewing procedures for GMO safety for submission to governments of the US, Canada, Argentina, Brazil, the EU, India, South Korea and Taiwan and reviewed hundreds of publications on allergenicity, toxicity and potential horizontal gene transfer. In my career I have not seen any documented cases of adverse health problems in humans or agricultural animals caused by consuming approved GM crops and I believe that the safety assessment of GMOs is quite robust[2-3].

Of course I had to go through a learning process to gain an understanding and comfort level with the assessment process for GMOs because I am a born skeptic. My scientific career began during the early years of development of agricultural biotechnology. This paper reviews some of the history of development of the safety assessment and regulation of GM crops in the US. It includes the primary proven food safety hazards and risks and describes the process of evaluating safety of new GM crops prior to commercial release. It includes a description of the most significant case of a GM product that was approved and then withdrawn from the market because of uncertainties of safety data, not because of harm.

1.1 Real risks of foods vs. hypothetical risks

Many of the foods we eat today were initially consumed hundreds to thousands of years ago. The genes and exact nutritional composition of many crops have been changed from the earliest varieties using conventional breeding techniques. However, to a great extent commodity crops including wheat, rice, corn and soybeans as well as many of the fruits and vegetables are quite similar to the food materials humans have consumed safely from these plants for centuries. The experiences of using those crops have guided regulators in establishing a safety evaluation process that begins with considering whether humans have had experience and contact or consumption of the host plant (the gene recipient) and the donor organism (source of the gene to be transferred).

In the mid-1970s as I was earning a bachelor's degree in biology I was an active member of Greenpeace, Friends of the Earth and Union of Concerned Scientists. Details of techniques of recombinant DNA methods were first being described in college classrooms as we learned about potentially useful recombinant bacteria and plants that might come from the technology. At that time most students and many professors had a very superficial understanding of DNA, RNA, ribosomes and protein synthesis compared to our knowledge in the 1990s and certainly compared to information available even in high school classes in 2014. In the early 1970s Paul Berg, Walter Gilbert and Frederick Sanger (all future Nobel Laureates in chemistry) began discussing potential (hypothetical) risks that recombinant organisms might pose if certain viral DNA sequences from pathogens were introduced into bacteria using this technology. Maxine Singer and others called on the community of scientists to develop safety standards. Much of the concern was on the proposed use of the simian virus 40 (SV40) DNA elements in recombinant bacterial plasmids that were being transferred in culture into monkey cells to understand gene function as described by Cole et al[4]. In response Berg and others organized the Asilomar Conference in 1975 at the urging of the National Academy of Science (US) to establish guidelines for ensuring safety. The process and twenty years of experience of safety of recombinant DNA work since then were reviewed by Berg and Singer[5]. Essentially all recombinant DNA work was halted in the US for one year while the guidelines were developed. They detailed considerations based on perceived risks and

called for the establishment of institutional biosafety committees to review each new rDNA experiment in any institution or company that was performing genetic engineering research. The primary focus was the potential risk or safety of the new DNA elements based on mode of action and risk of the DNA donor organism. The guidelines have helped ensure that really hazardous organisms were not created using the technology. Relatively safe cloning experiments can be performed in a typical clean laboratory environment with few restrictions (Biosafety level 1 or 2). There are few places with extremely tight controls (Biosafety level 3 or 4) where recombinant experiments can be performed on highly lethal and infectious agents (http://en.wikipedia.org/wiki/Biosafety_level).

The safety issues related to foods derived from GM plants are of course different. Genetically modified plants are not infectious, potential risks of food safety for GMO are quite low compared to risks from microbes and risks are not different from those posed by non-GMO plants. There are of course specific risks from foods that must be evaluated such as the potential transfer of an allergen or a toxin from another organism into a food crop. Many hypothetical risks are the focus of discussion today rather than the finite and definable risks that should be evaluated based on our extensive knowledge of science and safety. The evaluation of a new product that has added one or a few new gene(s), new protein(s), or new metabolites to a crop that has 10 000 to 20 000 endogenous genes and has already been safely consumed should focus on the safety of the gene source, protein characteristics and metabolites if the protein is an enzyme. The risks would be presented by the other 10 000 plus genes and proteins would be the same risks that already occur from that crop. In addition, the types of risks the new gene and protein could present are definable based on our experiences with other foods. Most current non-GM food crops have specific allergenic proteins; a few may have toxins (solanine) or anti-nutrients (trypsin inhibitors). So the focus on the new proteins should be on evaluating potential allergenicity, toxicity and any anti-nutritional properties.

There is now a history of nearly 20 years of production and consumption of a few commonly grown GM crops, for example insect protected corn containing a specific protein from Bacillus thuringiensis or Bt-corn; herbicide tolerant soybeans with a gene from a soil bacterium and virus resistant papaya, without evidence of harm. Crops improved through biotechnology have shown benefits due of reduced pesticide applications or in some cases reduced plant pathogen impacts. A number of GM crops have improved agricultural practices in ways that minimize soil erosion, energy or water consumption.

Some might argue that the strong fears voiced against GMOs stimulate healthy debates about proper regulatory studies that have helped ensure a robust assessment process. Others suggest that many of the new regulatory demands developers face today are excessive and delay scientific progress in medicine, industrial development and agriculture. The truth probably lies between the extremes, but based on conversations with GM developers, commodity companies and food companies as well as review of regulatory guidelines of the EU and other countries it is clear that the global process of GM evaluation and approvals are slowing development and leading to global trade barriers over the past 10 (2004 to 2014) years. Because of the international nature of trade, agricultural companies have to wait many years before new products can be released in order to obtain approvals in the major world markets. It seems that regulators in all countries are becoming more precautionary as they are afraid of being blamed for approval of a GM crop that is not proven to be absolutely safe under all possible uses. The precautionary principle is counter to the policy of the Food and Drug Administration (FDA) of the US as outlined in 1994, which recognized that all foods pose some risks that can be evaluated and managed and that the standard of safety is that foods from GM crops must be as safe as conventional crops of similar types.

A searching of scientific literature today identifies many new study questions and designs that are being performed on potential GM crops using a variety of search terms (transgenic, GM, genetically engineered, toxicology, reproductive, cancer) that should only be performed if there is as testable hypothesis based on information about the crop or the gene and gene products. Few (if any) dietary proteins alter reproductive fitness, cause cancer, act as adjuvants or increase the prevalence of a broad range of autoimmune diseases.

Today regulators and politicians are being pressured by activists like Eric-Gilles Seralini, Terje Traavik, Vandana Shiva, Mae-Wan Ho and Jeffrey Smith as well as celebrities like Oprah Winfrey, Dr. Oz and Cui Yongyuan or by consumers who listen to these activists make unsubstantiated claims of health risks of GMOs on websites in books, in the news media and television. For example, Jeffrey Smith's website, the deceptive

"Institute for Responsible Technology (http://www.responsibletechnology.org/) claims that very diverse human diseases including autism, celiac disease, food allergies and cancers are dramatically increasing due to increased consumption of foods produced from GM crops. He takes small observations from a few poorly controlled animal studies that have not been validated to predict human disease and implies that humans will experience many complicated diseases from eating foods derived from GMOs. Mr. Smith offers a training program for "anti-GMO speakers" for a fee of $150 USD. Mr. Smith does not post credible peer-reviewed scientific studies to support his claims and generally cites correlations of increased GMO production and increases in these diseases that have highly diverse and uncertain causes. In fact the correlations usually do not match the introduction of most of the GMOs in the food chain. Yet many highly educated people take statements by Smith and other activists to be factual and they refuse to look more deeply for the many public and published studies that are available to demonstrate the approved GMOs have been evaluated for safety by scientifically sound studies. There are no studies that link consumption of insect-protected corn to celiac disease or food allergies, nor autism nor cancers. If coincidental changes in our lives and environment demonstrated causality; we should stop air-travel, shut off the internet, discard cell phones and television; ban processed foods, vaccines and prescription medications. We would need to live our lives as they were in 1914 when the world population was less than two billion, life was very different and the average life-expectancy less.

In considering risks from foods, it is highly doubtful that genetic diversity of our foods represents a food safety risk. We are omnivores and subsist on highly diverse diets. We consume foods that are markedly different in 2014 compared to those consumed in 1914 and certainly compared to 1514 before tomatoes, potatoes and peppers were transferred from South America to Europe, India and China. If there are significantly different risks associated with eating plants that have only minor genetic differences compared to the varieties we eat every day, then maybe we need very complicated testing methods. However, humans have been pretty good at evaluating food safety over thousands of years without highly complex scientific studies. One could argue the extended life-expectancy, relatively low infant mortality rates and general health status of humans in the US and China in 2014 provides pretty convincing evidence that the current GMOs are not likely to be harmful. It is important to focus on realistic risks of foods and the development of processes that help ensure that foods produced from GM crops are as safe as foods produced from similar non-GM crops.

1.2 Early development of the safety evaluation of GMOs

In the mid-1980s I had not considered the safety assessment process that might be performed on GM crops to evaluate food safety. I knew little about the process of using agrobacterium mediated transformation system to insert functional segments of DNA into plants[6]. As I learned more about biotechnology during training as a PhD student at the Ohio State University, cloning a cDNA of bovine lactoferrin for sequencing and expression I had to learn and comply with evaluations by institutional review committees at the university. I had to answer questions about the source of the gene, the encoded protein, the plasmid vectors and the host cells and organism that was to receive the cloned DNA. The training was reinforced during my work developing cDNA clones for rodent and human cytokines as I studied immunology at Cornell University and later at the University of Michigan. By the time I joined Monsanto as a regulatory scientist working on the safety assessment of GM plants in 1997, I stopped believing the statements by Greenpeace and others about many hypothetical risks of GM crops and statements that there were no safety evaluations and resigned my memberships in those organizations. Within two months of joining Monsanto I was thrust into the role of developing an animal model to evaluate the potential impact of a GM event to evaluate potential impacts on allergenicity. The tests were novel and unprecedented as no one had demonstrated that a rodent model could predict potential sensitization in humans. However the government of India demanded an animal model test for allergenicity. The approval process took nearly 7 years after the US had approved the same crop. India dropped the requirement to use animal models to evaluate potential risks of food allergy after bringing their guidelines into alignment of the Codex Alimentarius Commission (2003) guideline[1] in 2008. My work at Monsanto involved becoming familiar with the regulatory process in the US and other countries and learning the science of risk evaluation for potential allergenicity, toxicity and nutritional equivalence. I continued being involved in the regulatory evaluation process when I was hired at the University of Nebraska in 2004 and have become even more broadly involved through 2014. But I am still learning about the process

that led to the current assessment.

A review of publically available information shows that academic, industrial and government scientists have collaborated in many consultations to develop a useful and predictive safety assessment process for GM crops. The US government outlined a coordinated regulatory framework in 1986 that includes the Food and Drug Administration (FDA) the Environmental Protection Agency (EPA) and the US Department of Agriculture to evaluate and regulate GM crops (Office of Science and Technology Policy, 1986; http://www.aphis.usda.gov/brs/fedregister/coordinated_framework.pdf). That was eight years before the first GM crop approval. A group of academic and industrial scientists held meetings as the International Food Biotechnology Council (IFBC) in collaboration with the International Life Sciences Institute (ILSI) and developed a risk assessment guideline that was published as a supplement to volume 12 of Regulatory Toxicology and Pharmacology (1990)[7]. The IFBC-ILSI volume was prepared by 28 highly experienced scientists and legal experts. The volume presented methods of genetic modification, variable crop composition of traditional foods, safety evaluation of food ingredients derived from microorganisms, safety evaluation of single chemical entities, safety evaluation of whole foods and complex mixtures and legal and regulatory issues. The draft reports were reviewed by 150 experts in industry, government and academia from 13 countries prior to publication. The major issues were presented and discussed by 120 experts in an open symposium. The IFBC-ILSI document presented a number of key evaluation steps and decisions for whether further evaluations were necessary and also discussed the legal food safety regulatory framework in the US. They supported the decision by the US government that foods derived from GM products could be efficiently regulated within the existing regulatory framework as they found that generating the new varieties (e.g., transformation through biolistics or *Agrobacterium* constructs) were not different in terms of potential impacts on safety compared to traditional breeding methods. The panel concluded the focus should be on questions related to characterizing and evaluating the safety of the introduced DNA, proteins and any metabolic products of any new enzyme in the GMO.

1.3 US regulatory process for GMO evaluations

In 1992 the FDA issued a policy statement on the safety and evaluation process for foods derived from new plant varieties including those derived from recombinant DNA techniques under the Federal Food, Drug and Cosmetic act (FDA Federal Register vol. 57, No. 104, docket No. 92N-0139). The evaluation process was followed for the safety assessment of the first GM crop approvals in 1994-1995 and although more complex now, are consistent with the process followed in 2014. Under the unified regulatory system the US Department of Agriculture (USDA) Agricultural and Plant Health Inspection Service (APHIS) is responsible for oversight of regulated field trials of unapproved GM events, control through a permit system of GM organisms, plant pests and veterinary products. A different section of USDA, the Food Safety and Inspection Service (FSIS) is responsible for regulating the safety of meat and some poultry products. The FDA has authority of other food safety issues including evaluating the safety of GM crops and all milk and dairy ingredients. The Environmental Protection Agency (EPA) is the lead agency involved in evaluating GM plant incorporate pesticidal (PIP) genes (e.g. plants containing genes encoding crystal proteins from *Bacillus thuringiensis*, or Bt plants; plants including genes for viral resistance such as the Plum Pox Virus resistant plum tree) as well as regulating chemical herbicides and chemical insecticides. The EPA and FDA follow the same food safety guidelines and the normal process for a PIP includes consultations with the FDA and a full dossier submission to the EPA. Although the FDA consultation and data submission is in theory "voluntary", failure to consult with FDA and provide data to complete evaluation of potential allergenicity, toxicity and nutritional effects of a GM crop is likely to lead to mandatory recall and legal action if there is any suspicion of harm. Requirements by EPA and USDA are clearly mandatory. Both the EPA and the FDA expect similar evaluation processes and tests for food safety before a product goes to market.

1.4 FDA policy on food safety of GMOs: as safe as similar varieties of non-GMOs

The FDA and regulatory agencies from Australia, Brazil, Canada, Japan, the Netherlands and the United Kingdom governments were significant contributors to the Codex 2003 guidelines[1] that were established as part of the Codex system that is agreed to by the US and China. The process includes evaluation of the same types of risks presented by non-GMO sourced foods that are known to cause adverse health effects: food allergy, food toxicity and adverse nutritional effects including potential increases in anti-nutrients or inclusion of potential celiac eliciting proteins (glutens from wheat and near-wheat relatives). Developers

are expected to present documented information evaluating the history of safe human use (HOSU) or exposure of the gene source and protein or gene, as well as information showing adverse effects. The information must include characterization of the gene products (protein or RNA) and any metabolites of any introduced enzyme, dose of consumption of the protein or metabolites that will be expressed in the new GM plant food material based on consumption patterns of foods made from the host organism. If there is historical evidence showing potential risk from consumption of the gene donor, additional testing may be required.

The FDA recognized that a few endogenous ingredients of all foods pose some risks for consumers. Some risks are normally mitigated by food storage, preparation (cooking) or limiting consumption. For instance lectins, protease inhibitors and amylase inhibitors of legumes (beans) are inactivated by cooking prior to consumption. Cassava is soaked and pressed to remove hydrocyanic acid to prevent cyanide poisoning before manioc is made and consumed. Potato varieties are selected in breeding to ensure they have low concentrations of the glycoalkyloid solanine as it is a mild toxicant. Young, green potatoes are not consumed as the content of solanine is high at that stage. Humans have adapted the foods and processing to ensure safety. Those hazards affect essentially all consumers if not handled appropriately. Other hazards that affect everyone are from contamination by bacteria, fungi or chemicals.

It is important to recognize that the most common and severe risks of food ingestion are from contamination of food with exogenous materials. Contamination can occur on the farm, or during storage in restaurants or homes. Bacteria, viruses, fungi, parasites and chemicals including mycotoxins, heavy metals and pesticides are relatively common food contaminants. The most significant acute risks are presented by bacteria including *Escherichia coli* O157 and other toxin producing strains; *Listeria monocytogenes, Salmonella* sp., *Campylobacter* sp, and *Clostridium perfringens*. The Center for Disease Control (CDC) and USDA FSIS estimate that there will be approximately 3 000 deaths in 2014 in the US population of 310 million, and approximately 128 000 hospitalizations (www.foodsafety.gov/poisoning/causes). Some parasites are also commonly spread through food. Toxoplasmosis is caused by Toxoplasma gondii, the most common food borne parasite in the US causing hospitalization and some deaths. Some viruses are commonly spread through foods. Norovirus is the most common cause of acute gastroenteritis in the US. It is spread through contact with many foods due to unsanitary food handling in a given outbreak, but rarely causes fatalities. Hepatitis A can lead to death in susceptible individuals who go untreated. Mold contamination is rarely documented as a cause of significant food borne illness in humans with the exception occasional outbreaks of mycotoxin poisoning caused by moldy grains[8]. However, mycotoxins more commonly cause severe outbreaks in poultry and other agriculturally important species as they are often fed grain at high concentrations[8]. Mycotoxins are small to moderate molecular weight organic compounds that are typically polycyclic and are not easily detoxified by the liver of some individuals or species. A few of the substances that cause toxic reactions are proteins, such as botulinum which is produced by the bacteria *Clostridium botulinum* along with some other toxins while ricin from castor beans is one of the rare plant protein toxins known to affect mammals[9]. Interestingly GM plants expressing plant incorporated protectants such as Cry1A in corn can reduce fumonisin (a mycotoxin) levels, thus reducing the potential for toxicity in chickens, pigs and cattle. Future GM products are likely to include specific anti-fungal and anti-microbial proteins that will further enhance food safety.

While toxins and anti-nutrients often affect nearly every consumer, a few hazards in foods only affect a small percentage of the population. Specific food allergens affect less than 1% of the population, but can cause severe reactions or death in a very small percent of the population. Glutens (gliadins and glutenins) of wheat and closely related grains cause celiac disease (CD), a chronic autoimmune disease in less than 1.5% of the population. Celiac disease affects a genetically restricted subset of the population that includes over 25% of the total population and there are many other factors that are not completely understood. A major focus of the food safety assessment then is to evaluate and ensure that the transfer of a gene into a GM plant does not transfer an allergen or a CD eliciting gluten from the allergenic or CD eliciting source into another source.

1.5 Recognized risks of food allergy including celiac disease in non-GM crops

The most common endogenous risks of food consumption are IgE mediated food allergies[10] and cell-mediated celiac disease[11-13]. Food allergy to all sources may affect 2% to 6% of the population in the

US, with varied degrees of severity. Individuals are usually sensitive to between one and five allergenic foods. Allergens pose a significant risk to those who are already allergic to the specific proteins while they do not pose a risk for non-allergic consumers. Because food allergy is highly variable between subjects in terms of severity of disease and the complexity of food composition, the sources of allergy for an individual are not always obvious[14-15]. The methods used for diagnosing food allergy are not standardized in many medical facilities and few doctors are well trained to accurately diagnose food allergy[16-17]. Food allergies are specific because the patient has been sensitized and produces IgE antibodies that bind specifically to one or more proteins in the food. In IgE mediated food allergy, reactions occur because the individual has developed specific IgE antibodies to at least two epitopes (IgE binding sites) on a relatively abundant protein in the food. Their IgE antibodies are bound to FcεR1 receptors on the surface of mucosal mast cells and blood basophils. Upon subsequent ingestion of the food containing the allergenic protein, the protein or fragments of the protein are absorbed and bind IgE on the mast cells or basophils, stimulating signals within the cell. If a sufficient number of allergen-IgE binding events occur within a few minutes it triggers the release of histamine and leukotrienes from the mast cells and basophils, inducing vascular leakage and symptoms due to angioedema and nerve stimulation. Some individuals experience relatively mild oral itching and mild swelling (angioedema) in the mouth and throat, others get hives or urticaria. Some experience asthma with wheeze and shortness of breath. Others may vomit or have diarrhea. A few will experience hypotension (drop in blood pressure). Anaphylaxis is a severe, life-threatening systemic reaction that includes hypotension and breathing difficulty that usually requires immediate medical attention including injection of epinephrine, other medications and oxygen. Perhaps 150 to 200 highly allergic individuals in the US die each year due from anaphylaxis triggered by food allergy[18]. Most who died because they did not receive immediate medical treatment including an injection of epinephrine. Peanuts, a few tree nut species, milk and eggs are the most common causes of fatal anaphylaxis from food[19]. Although exposure to the allergen triggers an acute reaction in the allergic individual, once sensitized, the individual may remain allergic throughout their life. However, young children often become tolerant to their allergenic food (milk, soybeans or egg) five or more years after initial reactions through a process leading to immune tolerance.

Estimates of the prevalence of food allergy are approximations. The best estimates available for the US, Europe and Japan indicate that food allergy affects from between 1% and 2%, up to 10% of the general population in those countries[20-21]. The frequency of cases of severe, life-threatening reactions is not well established, but clearly some allergenic foods such as peanuts, some tree nuts, cow's milk and eggs account for more severe reactions than fruits and vegetables. In most countries including the US there has not been a standard reporting system for food allergy anaphylaxis. Epidemiologists at the US Centers for Disease Control reviewed hospital coding within the US system for a period of 1997-2007 using various resources and estimated that there are approximately 317 000 food allergy related hospital visits per year in the US (years 2003-2006), with more than 9 000 admissions due to severe reactions[22]. Anaphylaxis was usually attributed to peanuts, crustacean shellfish (shrimp), tree nuts, milk, eggs and fish.

Celiac disease is a genetically restricted autoimmune disease initiated by sensitization to specific wheat, barley and rye glutens (gliadins and glutenins) by activation of T helper 1 type CD4+ T cells[23]. The disease is chronic and lead to flattening of the villi in the upper small intestine, wasting disease and sometimes to specific cancers and other autoinmmune diseases. The genetic restriction is due to unusual protein sequences that are presented most effectively by those with Major Histocompatability Complex loci HLA-DQ2.5 or HLA DQ8[24]. However, while more than 25% of the US population has either HLA-DQ 2.5 or DQ-8, only an estimated 1% of US consumers are clearly diagnosed with CD, which is similar to the rate in Europe[25]. The rate of CD in China is not known, but one recent study suggests that it is more common than once believed[26]. There are uncertainties in prevalence due to the complexity of accurately diagnosing affected individuals as endoscopy with multiple biopsies are taken as the gold standard following consumption of glutens, but endomesial-specific or tissue transglutaminase-specific antibody tests in conjunction with HLA typing or associations with diagnosed near relatives are often used as sufficient evidence for diagnosis[27]. Specific peptides of glutens and gliadins have been identified as stimulating Th1 CD4+ T cell clones from MHC-restricted CD patients[28-30]. The only way CD patients manage their disease is through avoiding consumption of foods containing proteins from wheat, barley, rye and for some, oats[31]. There is

also a growing number of consumers who believe they have non-celiac gluten sensitivity, however the specific disease pattern is not uniform, the mechanism of reactions are not known and there is some disagreement between Gastroenterologists as to the authenticity of the disease[32].

1.6 Food allergens are specific proteins, not whole foods

Generally people describe food allergy as being a reaction to a whole food (e.g. milk, eggs or peanuts). But research over the past two decades has identified specific proteins in the foods as the causes of allergy. The International Union of Immunological Societies (IUIS) Allergen Nomenclature Subcommittee (www.allergen.org) lists 12 protein families as commonly allergenic. The most prominently named peanut proteins reported as the dominant allergens are the small molecular weight prolamins (14 to 18 kDa) including the abundant 2S albumins (Ara h 2 and Ara h 6) and higher molecular weight cupins (50 to 75 kDa) major seed storage proteins, Ara h 1 and Ara h 3. The cupins each account for more than 15% of the total protein content of the seeds. Subjects with substantial IgE concentrations to any of these four proteins are most at risk for severe reactions following ingestion of peanut[33-34]. The Ara h 2 and 6 proteins are highly cross-linked small molecular weight proteins with four intra-chain disulfide bonds making them relatively resistant to digestion by the stomach protease pepsin[35-36]. A few other proteins have been identified as allergens in peanuts but represent low abundance and/or low stability proteins which are considered to be minor allergens. Most people with clear IgE mediated allergy to peanut have IgE to the major allergenic proteins, it is not clear that IgE to the minor allergens cause significant clinical reactivity. A few proteins in some foods are nearly identical to homologous proteins in other foods or in pollen and are considered pan-allergens since the IgE of one subject may bind the homologous proteins from a wide variety of species. The pan-allergens do not cause serious reactions in most allergic subjects. Pan-allergens in peanuts include profilin (Ara h 5), pathogenesis related protein-10 family members (Ara h 8.0101 and Ara h 8.0201) and a lipid transfer protein (LTP), Ara h 9. The sequences of two defensin proteins Ara h 12 and Ara h 13 recognized by the IUIS nomenclature committee has not yet been published and the frequency and severity of induced allergic reactions are unknown. Individuals allergic to tree nuts including almonds, hazelnuts, pecans and walnuts usually have IgE antibodies that recognize similar 2S albumins and cupin seed storage proteins. In some cases there seems to be cross-reactivity among the tree nut proteins and even to peanut, but it is difficult to separate IgE cross-reactivity from de novo sensitization, where a subject is co-sensitized and co-reactive. Certainly though pecans and walnuts are very closely related and their allergenic proteins nearly identical.

A number of individual IgE-binding allergenic proteins from foods, inhalation sources (pollen, house dust mites and mold spores) and dermal (latex) or injection (venom, saliva of biting insects) sources have been characterized and studied in the past 25 years. The sequences of the proteins with published proof of IgE binding using sera from appropriately allergic subjects have been included in the AllergenOnline.org database managed by the Food Allergy Research and Resource Program at the University of Nebraska-Lincoln (www.AllergenOnline.org) to provide a bioinformatics tool for the GM safety assessment process. A number of the proteins included in the Allergen Online database have also been demonstrated to cause biological reactivity by skin prick tests of allergic subjects, basophil histamine release or basophil activation and those proteins are more reliably defined as allergens. The references used to categorize each allergenic protein group are listed in the database (www.AllergenOnline.org) along with an explanation of the process of classification. The database also provides sequence comparison algorithms to evaluate potential new GM or novel food proteins for potential risks of cross-reactivity.

It is not clear why people become allergic to certain proteins and foods rather than becoming tolerant to these generally innocuous proteins although there are genetic risk factors for IgE mediated allergy. It is clear that the prevalence of food allergy is rising in industrialized countries and it cannot be explained by changes in the genetics of consumers[37]. There are a number of proposed mechanisms including the "hygiene" hypothesis (lack of certain bacterial types from the environment or within the gastrointestinal tract) for sensitization (induction of specific IgE) and tolerance (suppression of IgE and allergy), but no single markers or hypothesis fits everyone[37]. Very likely multiple factors interact at the time of introduction of foods in the developing child, food processing methods, reduced vitamin D levels due to sedentary indoor lifestyles and reduced exposure to certain microorganisms or reduced parasite burden that together are contributing to increases in allergic

disease.

Celiac disease is elicited by a limited number of glutenins and gliadins from wheat, barley, rye and possibly oats, all members of the Pooideae subfamily of grasses. In order to provide a possible risk assessment tool for food safety assessment, we have gathered 1 016 peptides and 58 proteins f that have been found to stimulate CD restricted T cells into a CD-specific database for use in risk assessment (www.allergenonline.org/celiachome.shtml). We have also developed bioinformatics tools that help evaluate novel food proteins for identity matches to be able to flag potentially important proteins as possible risky proteins for those with CD to consume.

2 Assessment of GM crop safety in the US

2.1 History of safe use (HOSU)

The scope of the HOSU evaluation of the gene source, the gene recipient and the specific products of the gene includes determining whether there is documentation of direct contact with the protein or indirect contact with metabolites if the protein is an enzyme. Descriptions of appropriate allergenicity and toxicity assessments have been published by experienced scientists who have expertise in those areas[9,38]. In cases where the gene source is a common cause of allergy or toxicity, additional tests are likely to be required compared to sources without any history of allergy or toxicity. For example, peanuts and certain tree nuts (walnut, pecan, almond and hazelnut) are considered common causes of allergy. If a gene is transferred from one of the commonly allergenic sources, specific serum IgE testing is likely to be required similar to the study performed by Nordlee et al., for the Brazil nut 2S albumin[39]. If the gene source is castor bean (*Ricinus communis*), the *Closteridium botunlinum* bacterium or a wasp (*Vespula germanica*), regulators are likely to ask for additional specific toxicity tests to verify that the protein is not a toxin. Specific testing requirements will be dictated by the nature of the risk. If the source has neurotoxicity, then neurotoxicity tests are likely to be called for. The identifiable risks of the source would normally be discovered by searching published peer reviewed literature, although sometimes sources including searching Google may be useful. If there is a clear history of consumption of the source material, and the protein in question is proven to be expressed in the material that is consumed (e.g. the nut, fruit or herbaceous material), the lack of allergenicity or toxicity would aid in determining the protein is unlikely to present a risk. However, in many cases there will not be a history of safe consumption, which does not automatically mean additional tests are required, only that there may be slightly less certainty of safety.

Often there are clear, restricted risks associated with a given gene source. Apples contains two proteins that might be considered significant allergens, a non-specific LTP that is known to cause severe allergy in a very small number of consumers and a less potent, common cross-reactive protein Mal d 1. The Mal d 1 protein is a sequence similar homologue of an airway allergen Bet v 1 that is common in pollen of birch and related tree species. Other proteins from apple are expected to represent low or no risks of food allergy. Peanuts contain four potent allergens and a few additional minor allergens. Food labeling laws are written to differentiate risks of food allergy based on the prevalence and severity of allergy to the sources. In the US, Europe and Japan, peanuts are considered common and important sources of food allergy and any processed food that contains an ingredient from peanut must be labeled as to source. Apples are not considered to be common, potent sources of food allergy. The safety of proteins derived from a peanut gene would be more thoroughly evaluated than a protein from apples for potential risks of allergy.

The source of the insecticidal crystal proteins Cry1A, Cry2A and Cry3A is the bacterium *Bacillus thuringiensis*. Spores of this species have been used as microbial pesticides for 70 years without demonstration that they cause allergies or toxicity in mammals. The historical safe use of the organic pesticides provides assurance of HOSU for some Bt toxins, although that is true only for proteins that are demonstrated to be expressed by the bacteria used as microbial pesticides and not from all varieties of the species.

The developer is expected to provide documentation of the history of safe use of the gene source organism and if possible of the gene products. The description should also include evidence that the protein or other gene products are expressed in the materials encountered in food as well as a description of preparation of the food.

2.2 Characterizing the new protein and product attributes

The developer must describe the DNA or RNA sequence transferred in making the GMO. The source of other genetic elements (promoter and terminator) in the construct must be included. The method of transfer must be defined. Confirmation of copy number,

gene integrity and stability of the DNA through reproductive cycles of the organism must be verified. Any gene product should be quantitatively measured under conditions of normal use of the plant. In some cases mRNA size and accumulation in various plant tissues are also necessary to ensure the transcript is as expected. In most cases the gene encodes a protein. If the protein is an enzyme, any expected and measured metabolites must be described. The function of the gene and products must be disclosed. The sequence of the DNA and the protein are disclosed and data comparing the protein amino acid sequence to known toxins and allergens must be evaluated.

2.3 Potential allergenicity

Due to the importance of food allergies, the FDA has focused on preventing the transfer of allergens into a new food source as a primary concern for GM crops. A major risk for consumers with allergy to peanuts would be the transfer of a gene encoding a major peanut allergen into rice or corn. That possibility was demonstrated by the experience of Pioneer Hi-Bred when they transferred a gene encoding the 2S albumin from Brazil nut into soybean to improve feed quality for animals. Soybeans have a high concentration of protein, but are deficient in sulfur containing amino acids. The 2S albumin of Brazil nut is a small protein with a high concentration of methionine and cysteine amino acids. Pioneer Hi-Bred was preparing a dossier for submission for regulatory review for this potential product when they consulted with Dr. Steve Taylor at the University of Nebraska who suggested that since Brazil nut is known to cause food allergy in some consumers, the protein expressed by the transferred gene should be evaluated for potential allergenicity. In 1995 no one knew what the allergenic proteins were in Brazil nuts, but during studies described by Nordlee et al.[39], it became apparent that the 2S albumin is an important allergen. The results were published and Pioneer Hi-Bred stopped development of that potential product without submitting it to regulators. The experience helped validate the evaluation process that had been outlined in the FDA Federal Register in 1992. The experience also helped crystalize the evaluation process outlined by Metcalfe et al.[40], for evaluating potential allergenicity of GM proteins and eventually the Codex Alimentarius Commission guideline first published in 2003[1].

Food allergy is usually restricted to reactions mediated by antigen specific IgE antibodies and the mechanisms described can be found in any immunology text book. Most dietary proteins stimulate the immune system to become tolerant to contact with the protein. However, for those prone to allergies, their T helper cells and B cells may become educated to develop IgE immunoglobulin production because of the mixture of cytokines and cell surface signals provided by T-helper type 2 cells. The B cells differentiate into plasma cells or B memory cells expressing high levels of protein-specific IgE that becomes bound to the FcεRI high affinity receptors on mucosal or dermal mast cells and blood basophils. When the antigen is absorbed again in subsequent meals, it cross-links IgE antibodies on the receptors if at least two epitopes are bound and initiates a signal cascade. If a sufficient number of cross-links occur within a few minutes the mast-cells or basophils releases histamine, leukotrienes and proteases that elicit vascular leakage and inflammation. Symptoms may include angioedema, urticarial, asthma, emesis (vomit), hypotension (drop in blood-pressure) and in rare cases death due to systemic anaphylaxis. Since the IgE antibodies are specific in peptide epitope recognition, the symptoms are reproducible; same antigen, similar reactions. Generally allergic sensitivity is assumed to be life-long. Many dietary proteins also induce IgG and IgA antibodies, but those are not risk factors for acute food allergy. Production of these immunoglobulins by B cells also requires T cell help, but the responses and signals differ from those leading to IgE responses. The focus of the allergenicity evaluation is therefore on measuring IgE responses.

There are also T-cell mediated reactions to some dietary proteins, the major one being gluten-sensitive enteropathy or CD as discussed previously. Evaluating GM proteins for potentially eliciting CD is relatively straight-forward and will be discussed later. There are rare cases of T cell mediated food protein induced enterocolitis syndrome (FPIES), which is a severe reaction primarily to proteins in cow's milk or soybean but occasionally to proteins in rice or oats and a few other foods[41]. Individuals usually become tolerant to the responsible food within three to five years and no specific proteins have been identified as the causative agents. Therefore it is not possible to evaluate proteins as a possible cause of FPIES at this time.

There is credible evidence that the prevalence of food allergies and celiac disease are on the rise globally, although there is great uncertainty about the magnitude of the rate of increase and the cause. Part of the increase is likely due to increased consumer awareness of allergy and CD as well as more awareness and testing by doctors. There is much misinformation about prevalence and people are often incorrectly

diagnosed. Many individuals reported being food allergic, but a clinical evaluation demonstrates they are not food allergic in many cases.

The major risk for food allergy is acute, within minutes to hours after consumption of the allergenic food. The primary risk of food allergy from GM crops is the potential transfer of a protein that already causes allergy in specific consumers. If affected individuals consume a biotech crop that includes their allergen, reactions would likely be as severe as they would be to the natural source of the allergen. Thus the primary concern for GM crops is to avoid the transfer of a protein that already causes allergy (of any kind, contact, airway or food) into a food grade plant of another species.

The International Life Sciences Institute (ILSI)-Allergy and Immunology Institute and the International Food Biotechnology Council organized discussions and a series of scientific peer-reviewed publications to consider potential risks of food allergy from GM crops. The publications were presented in a special issue of Critical Reviews in Food Science and Nutrition (Vol. 36, Supplement, 1996). Panelists included scientists with expertise in biotechnology development and regulation or allergens and allergy. The first chapters explain allergy, food allergy, the biology of plant proteins the process of genetic modification of food plants and review allergenic foods known at the time. Two chapters provide the basis much of the background information that guided development of a science based assessment process to evaluate potential risks of food allergy for novel proteins[42-43]. The last chapter outlines an evaluation process to determine whether a protein expressed by a transgene would potentially present a risk of food allergy to consumers[40].

The evaluation process outlined by Metcalfe et al.[40] was consistent with the FDA recommendations of 1992, and included decision tree flow-chart beginning with evaluating the allergenicity of the source of the gene. However, the decision tree did not exactly match the description in the text and some things were not clear. Fig.1 represents my interpretation of the tree from the text[40]. If the gene is from a clearly defined allergenic source (food, airway or contact allergen), the next step would be to obtain sera from 14 humans allergic to the source and test for IgE binding to the GM protein using standard laboratory test methods. If fewer than 5 allergic donors are found for the test, then the protein is evaluated for stability to digestion by pepsin. All proteins regardless or source should be evaluated by sequence comparison to known allergens and a list of known food and respiratory allergens known in 1995 was included[40]. They recommended using FASTA to align the protein to known allergens and search for any contiguous 8 amino acid segment having an identical match to any allergen. In practice

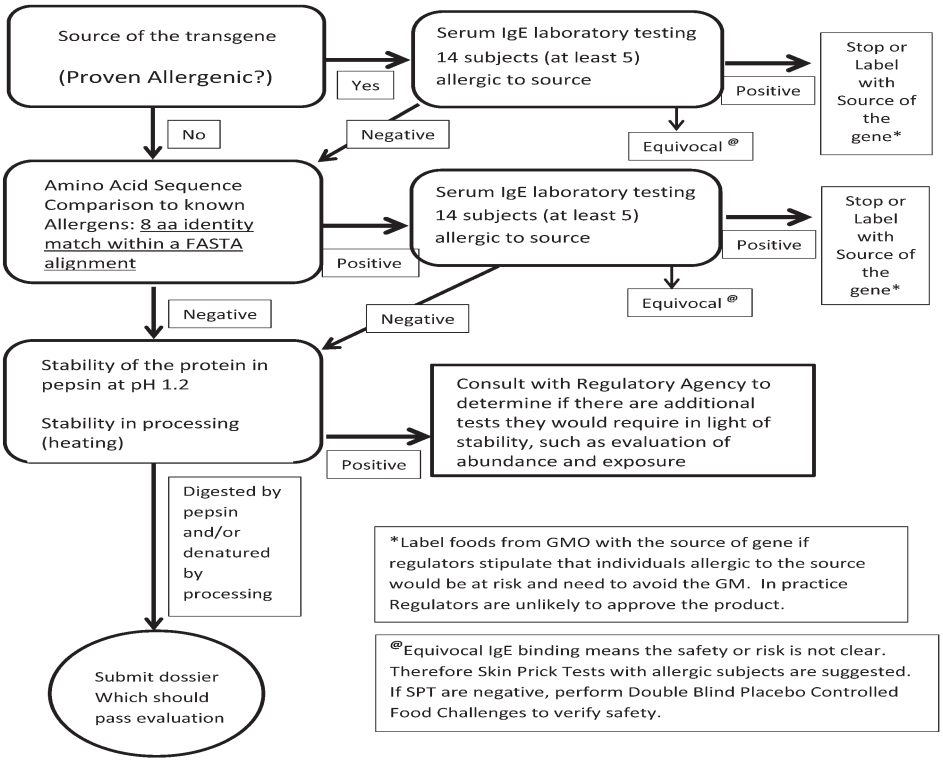

Fig.1 Assessment of the allergenic potential of GM proteins, adapted from Metcalfe et al., 1996 to more accurately reflect the description in the text.

most bioinformatics matches were simply performed by a sliding "WORD" match of 8 amino acids. If the protein matches an allergen, sera from humans allergic to the source of the matched allergen would be tested for binding. If IgE from appropriately diagnosed allergic serum donors clearly binds to the protein, the developer is likely to stop development and not submit the dossier to regulators. However, if they would try to continue, the regulator would likely demand foods made from that GM event would have to be labeled as to the source to alert allergic subjects to avoid the food. In the case of inconclusive IgE binding, subjects allergic to the source would be tested by skin prick tests (SPT) with the protein and if all are negative, they would be asked to undergo a double-blind, placebo-controlled food challenge (DBPCFC), under ethical panel approval. In addition, the protein would be tested for stability in acid with pepsin (protease) and the time of disappearance would be graded to evaluate digestibility. The protein might be tested following typical food processing methods for that specific crop to determine if it denatures. However, processing stability is really only useful to understand if a risk of allergy or toxicity can be mitigated by normal food processing, similar to the inactivation of natural lectins and protease inhibitors in legumes during cooking. If the protein is stable to digestion by pepsin, the regulatory agency would be consulted for any requests for additional tests.

Most GM events and newly expressed proteins approved in the US meet the criteria for minimum risks regarding the allergenicity evaluation presented by Metcalfe et al.[40] and the Codex[1]. A literature search finds only one potential GM crop product that did not fit the history of safe use and upon testing for serum IgE binding using samples from the at-risk population of Brazil nut allergic subjects was found to bind IgE[39]. That potential product was not submitted to regulators and was terminated by the developer (Pioneer Hi-Bred). No currently approved product that I am aware of received a gene from a commonly allergenic organism. Thus the Brazil nut 2S albumin is the only one that would have presented a major risk of food allergy to a subset of consumers.

2.4 Bioinformatics for matches to allergens

In addition to evaluating the source of the gene the bioinformatics search for identity matches between the GM protein and any known or suspected allergen has become probably the most important tool to identify possible risk and a reason to do serum IgE testing[38,44-45]. The Codex[1] document calls for a FASTA or BLASTP search with the amino acid sequence of the GM protein against a database known allergens. The www.Allergenonline.org database is the most comprehensive peer-reviewed allergen database that I am aware of. The criteria of greatest emphasis is any match >35% identity over any segment of 80 or more amino acids. The Allergen Online database (www.AllergenOnline.org) was established in the Food Allergy Research and Resource Program at the University of Nebraska in 2004 and implemented an expert review process. It is updated annually to provide a curated database and search algorithms for risk assessment of allergenicity using bioinformatics tools[44].Version 14 of the database was released in January, 2014 and includes 1 706 sequences from 645 protein-taxonomic groups representing 290 species. In my opinion the search for >35% identity over any segment of 80 amino acids is quite conservative as described in a number of publications. There is little evidence of in vitro cross-reactivity for proteins sharing less than 45% identity by overall alignment (full-length). And in terms of shared allergic reactions due to cross-reactivity, very few proteins sharing less than 50% overall identity matches are cross-reactive[46]. Since 1996 there have been a number of scientific consultations and recommendations for "improving" the allergenicity assessment of GM crops. The FAO/WHO expert panel review[47] suggested including a number of changes that were not validated. The FAO/WHO recommendation was to use FASTA or BLASTP to identify any segment of 80 or more amino acids with an identity match of >35%; and to search for short identity matches of six contiguous amino acids (aa) rather than eight aa suggested by Metcalfe et al.[40]. But those precautionary criteria have not been validated. They were reviewed in previous publications[3,38,45]. A number of studies demonstrated that searches for six aa identity matches produce far more false positive matches than true positives. The eight aa matches are better, but still do not have a high predictive value. Others have also described the 80 amino acid searches as being overly conservative; however I have not identified many probable false positives using that criterion, but also did not miss any likely cross-reactive protein pairs[45]. There is some disagreement about the optimum algorithm for the 80 amino acid search as some bioinformaticians suggest searching for the best overall alignment by FASTA or BLASTP and then scoring the best match as a more reliable alternative[48-50]. Recently the European Food Safety Authority (EFSA)[51] dropped the recommendation

to search for short, contiguous identity matches and the European Commission[52] accepted that advice in dropping it from their regulations. There have been a few instances where short contiguous identity matches that occur by chance (no evidence of overall sequence homology), have led to requirements for serum IgE tests that were not needed[53]. There is a risk in performing such tests as in vitro IgE binding results can be ambiguous, with false positive binding that may inappropriately implicate a protein as a possible allergen[54]. Although there is little relevance of IgE binding to a single short segment of two non-homologous proteins means a risk of allergy in that situation is unlikely, some regulatory bodies would want the developer to continue the investigation and possibly demand *in vivo* testing in humans.

2.5 Serum IgE testing

Serum IgE tests are rarely warranted for evaluating the potential allergenicity of GM proteins. However, if serum IgE testing is warranted the assays must be well designed, the methods should be validated with known allergens for the allergic serum donors and the test subjects should be demonstrated to have the appropriate IgE sensitivities. Test materials should include purified GM protein, purified allergenic target (e.g. the sequence matched allergen), and the specificity of any IgE detection antibody must be verified, appropriate blocking solutions are needed. In some cases specific inhibition assays may be required. Critical factors in materials and assay design are presented in a number of publications[45,54-55]. If the protein may contain asparagine-linked carbohydrates, there is the possibility the plant might modify the protein with the addition of alpha-1,3 fucose or beta-1,2 xylose on the stem of the asparagine-linked glycan may bind IgE from many subjects, but there is little or no evidence for clinical reactivity[56-57]. Those structures are known now as cross-reactive carbohydrate determinants (CCD). If there is a signal peptide and an N-linked sequon (Asn-x-Thr/Ser), the protein should be tested for the presence of CCD. Inhibition studies may need to be performed to evaluate the relevance of any *in vitro* IgE binding. If there is evidence of IgE binding in vitro and there is a desire to continue with development of the product, the biological relevance of binding may be tested using basophil activation or basophil histamine release[58]. Alternatively, skin prick tests (SPT) or double-blind, placebo-controlled food challenges may be required using highly characterized test materials and subjects who are well informed and have consented to the challenge.

2.6 Potential de novo sensitization: stability in pepsin and abundance

If there is no evidence the protein is likely to be an allergen based on source and a lack of bioinformatics match, there is no justification for performing serum IgE tests. There is a low probability of risk and there is no at-risk population. The only other questions regarding allergenicity are whether the protein might sensitize de novo. As suggested by Metcalfe et al.[40], proteins that are stabile in pepsin in an *in vitro* digestion assay and are abundant, have a higher probability of being an important food allergen. However, the correlation is modest even though many major food allergens are stable or fractions of the protein are stable and abundant[59]. The correlation of stability in pepsin has been performed at pH 1.2 and 2.0 and the FAO/WHO, 2001 recommendation was to use both conditions to evaluate stability. We did not find any significant difference[59]. The EFSA[57] recommendation was to use more "physiological" pH (3.5), but that has the effect of markedly reducing pepsin activity and has not been investigated in terms of predictive value. The FDA continues to accept the use of either pH 1.2 or 2. There is also not a consensus on abundance although it is clear that the abundance of a number major allergenic proteins in plants used for foods is greater than 1% of the protein in the food fraction[60].

Most of the GM proteins have been found to be digested rapidly in pepsin at pH 1.2 or 2. However, the Bt protein Cry9C that was originally introduced into corn to protect against the European corn borer moth larvae by Plant Genetic Systems (PGS) in Ghent, Belgium was found to be quite stable in pepsin. The product was called StarLink corn. The company was purchased by AgrEvo, then Aventis CropScience which was finally acquired by Bayer CropScience. Food approval was withheld because the protein was stable in the pepsin digestion assay (described later) and regulators felt there was some risk the protein might eventually sensitize someone, predisposing them to allergic responses to Cry9C. StarLink corn was grown on ~ 122 000 hectares in the US in 1999, and some grain from the corn was accidentally, but illegally included in some human food products (corn chips and taco shells). Tests by an anti-GM NGO discovered the inclusion of StarLink corn event in some food products and notified the US government and news media. Interestingly the question was whether people might become allergic to the protein, which would take time to sensitize people. There is no indication that people

were pre-exposed to Cry9C, so sensitization would have been from exposure in the contaminated taco shells and chips. However, within two weeks of the announcement more than 100 consumers complained they had experience food allergic reactions following consumption of taco shells or corn chips. Since corn is one of the least allergenic of grains and the quantity of Cry9C was quite low in corn grain and the grain was grown for only one year, it is highly unlikely that anyone was sensitized to the protein. However, the Center for Disease Control of the US investigated each consumer report. Those individuals who claimed reactions that might be consistent with food allergy were asked if they would provide blood samples and 18 did[61]. None of those individuals had IgE specific for Cry9C[61]. Since the grain and corn seeds were released and in food without approval, the US government demanded recalls and monitoring. Foods, ingredients and corn seed were screened and those containing the Cry9C protein or the transgene were pulled from the market. It took six or more years to completely remove all traces of Cry9C from seed and grain stores. There have been rough estimates that total costs for removal may have exceeded $500 million. Yet we should remember that there is no proof that anyone was harmed by consuming Cry9C. There is clearly a different level of risk of allergy that might be present from a GM food crop such as StarLink than would be associated with an outbreak of Norovirus, hepatitis or *E. coli* O157. We might conclude that the regulatory response was not in proportion to the risk in the case of StarLink. However, the ability to remove a GMO from production was demonstrated by the recall of StarLink corn and it shows that you can remove a GMO from the agricultural and food system if there is a reason to do so. It just takes time and an enormous amount of money.

Another product that is not as rapidly digested in pepsin as Cry1Ab (in corn) or CP4 EPSPS (in herbicide tolerant soybeans) are the two proteins (Cry34Ab2/Cry35Ab1) in another insect protected corn event. The proteins have intermediate stability as reported by Dow, the developer[62]. The EPA did allow this product into the market as the abundance of the proteins is low in grain and the stability intermediate.

New proteins expressed in the GM crops approved so far have been expressed and accumulate at low levels in the food materials of the crop, often in the range of or less than a few micrograms per gram dry weight of seed (CERA GM Crop database, 2014; http://cera-gmc.org/index.php/GMCropDatabase). Thus all of the GM proteins accumulate at levels markedly below the concentration of most of the important dietary allergens (typically >1% of total protein).

There is no published evidence that an approved GM crop has caused allergies due to the presence of the transgenic protein. A study was performed to determine whether soybean allergic subjects might have IgE binding to the CP4 EPSPS enzyme that was introduced into soybean to provide tolerance to the herbicide glyphosate[55]. This was not a regulatory study, but was performed as a stewardship study to see if there was any evidence of sensitization years after the product entered the market. Serum samples were collected from soybean allergic subjects in Europe and South Korea and tested using common protocols and highly characterized test materials. The study did not find evidence of IgE binding to purified CP4EPSPS or to the protein in extracts of GM soybeans[55].

2.7 Potential improvements for evaluating IgE mediated allergenicity

The FAO/WHO panel[47] recommended using targeted human serum testing in an attempt to determine whether a protein that is not similar to any known allergen might pose a risk due to existing sensitization or cross-reactivity. Targeted testing was defined as in vitro IgE binding tests using sera from 50 subjects with allergy to sources that are broadly related to the source of the transferred gene. For genes from a dicotyledonous plant, individuals allergic to one or more other dicot species would be used for serum testing. There was an exemption for proteins from bacteria since there are almost no allergies to bacteria. The targeted serum testing has never been tested in a way that would demonstrate its predictive power and it is counter-intuitive based on our knowledge of cross-reactivity. Homologous proteins from even moderately related sources (family level) are rarely cross-reactive by in vitro tests and clinical reactivity is rarely shared. The only proteins that are so broadly cross-reactive in laboratory tests are profilins, PR-10 proteins (Bet v 1 homologues), lipid transfer proteins and tropomyosins from crustaceans and other invertebrates. Those are all easily identified by bioinformatics. The US does not recognize targeted serum testing as a useful tool for the assessment of novel proteins.

The FAO/WHO[47] also recommended performing sensitization tests using two species of animal models, or two routes of sensitization in one species to evaluate the allergenic potential of each new protein. While many laboratories have tested various animal models in an attempt to predict the allergenicity of proteins, there

are none that have proven predictive over a wide range of effective allergenicity (from mild or non- to strongly allergenic), as reviewed by Ladics et al.[63]. There has been research that shows some promise for evaluating mechanisms of allergy and immunotherapy[64] and for preliminary ranking of allergenic sources[65-66]. A few have tested purified or partially purified proteins[67], but have not been validated to rank new proteins in the context of potency or prevalence of allergens in the human population[63]. The US does not recognize animal models as being useful at this time for predicting the allergenicity of novel proteins.

The Codex guideline did incorporate the recommendation for testing the sequences using the FASTA or BLASTP search alignments to identify matches of >35% identity over any segment of 80 or more amino acids. Codex[1] also retained the language suggesting the use of a short identity match of 6 or 8, but suggested the evaluator must justify that choice. The US regulators now expect a comparison for identifying matches with >35% identity over 80 amino acids using a comparison like that available on www.AllergenOnline.org or a full-length comparison by BLASTP with evaluation of alignments to meet the same criterion. They do not seem focused on short-8 amino acid matches, but most (all?) developers have supplied that data.

The EC regulation[52], which was based to a large extent on recommendations from another expert panel review process by the EFSA[51] also includes a number of suggestions for unproven tests including: evaluation of potential adjuvanticity of the new protein; the use of proteomics to consider possible changes in the expression of endogenous allergenic proteins for commonly allergenic species (e.g. soybean, peanut); and the use of more physiological pH (3.5) for the pepsin digestion assay. Yet those test methods have not been validated to demonstrate they would improve the risk assessment and are not asked for by US regulators. The US regulators do not ask for additional tests such as potential adjuvanticity unless there is information that would reasonably support the hypothesis that a new protein may be a lectin or have some other adjuvant-like properties.

2.8 Celiac disease

Risks related to CD have only been found to involve certain glutens (gliadins and glutenins) from wheat and near wheat grain relatives. Codex[1] recommends and the US government would require an evaluation if a gene from wheat, barley, rye or possibly oats, is transferred into another species, such as corn, rice, or sorghum. As far as I know, no developer has submitted a potential product to US regulators using such a construct. While the Codex demands an evaluation for proteins from wheat or wheat relatives, they have not provided guidance on the process. My laboratory considered the problem in the context of what is currently known about CD and the glutens involved in and developed a celiac database to provide a bioinformatics tool to allow rapid identification of potential hazardous proteins. In order to develop the tool we reviewed published scientific information on CD.

Symptoms of malabsorption and diarrhea associated with diet of bread were first described nearly 2 000 years ago in medical writings from Greece[68]. But it wasn't until 1888 that a physician in the United Kingdom (UK) gave the name coeliac (or celiac) to those suffering intestinal distress associated with eating foods containing wheat. Those observations were lost on modern medicine until 1952 that physicians in the UK published descriptions associating the wasting and intestinal pathology with the consumption of wheat. In the 1990's gastroenterologists developed methods for endoscopy and developed antibody tests that demonstrated patients with CD were developing antibodies that bound to connective tissue in the intestine and had T cells that were activated upon binding wheat peptides from glutens in the context of specific major histocompatibility antigen resenting receptors. Recent studies have identified many peptides from glutenins and gliadins of wheat, barley and rye grains that are responsible for activating T cells in genetically susceptible individuals[30, 69]. As many of these discoveries were occurring in the mid-1990's and beyond, the evaluation of proteins in wheat, barley and rye that might be responsible for causing the T cell specific responses in the fraction of subjects with the correct MHC Class II for susceptibility (MHC DQ 2.5 and MHC DQ 8) were just emerging. Since then many studies have been published that identified peptide sequences that are responsible for binding to the right MHC and activating effector T cells in those with CD have emerged. While the Metcalfe et al.[40] and the Codex[1] recommendations do not recognize the predictive capabilities of bioinformatics to evaluate risks of celiac disease from wheat subfamily proteins, it is clear that a substantial number of proteins were being identified that might serve as a database of "risky" proteins. Metcalfe et al.[40] and the Codex[1] both suggest that genes taken from wheat or wheat relatives that encode proteins should be evaluated for

their potential to cause CD, they did not specify how. In 2011, Plaimein Amnuaycheewa, a PhD graduate student in my laboratory reviewed more than 50 available publications identifying peptides involved in T cell reactivity using cell samples from celiac patients and we developed a database of peptides that can be used to screen potentially hazardous peptides from proteins from the wheat and wheat relatives. We have constructed a database of peptides from wheat, barley and rye that cause T cell stimulation or intestinal epithelial pathology (www.allergenonline.org/celiachome.shtml). The database is part of the www.AllergenOnline.org database for bioinformatics evaluation of potential IgE mediated allergenicity for GM proteins. Currently it includes 1 016 peptides with published evidence of T cell reactivity using cells from CD patients in the context of MHC Class II DQ 2.5 or DQ8, or toxic effects in intestinal epithelial cells or pathology in intestinal villi from those with CD. The amino acids of proteins introduced into GM crops may be searched for exact matches to the peptides in the database, or the proteins can be searched by FASTA for meaningful matches to 68 whole proteins known to stimulate CD, using criteria of >45% identity over alignments of at least 100 amino acids as potentially stimulating CD. A total of 53 references are included to explain the selection of peptides and proteins that might cause CD in susceptible individuals. Similar to the allergenicity assessment, bioinformatics methods should are able to identify proteins that might represent a modest to clear risk of causing disease. If there is a desire to introduce a wheat sub-family protein into another crop e.g. rice or eggplant (brinjal), the amino acid sequences there should be screened using this database to consider risk. If a positive match is found, the protein should be tested using cells or challenges in CD subjects to evaluate risks using cell based assays or possibly food challenges in at least 10 consenting CD subjects to ensure minimal risk to the CD population as the "at-risk" group of consumers. The bioinformatics criteria we believe is predictive based on extensive simulations is any 100% identity match to one of the 1 016 peptides or a FASTA match of >45% identity with any segment of 100 aa or more having an E score of < 1x 10-15. Genes taken from plants outside of the Pooideae subfamily of grasses represent little risk of causing CD and therefore even if they are homologues of glutens that cause CD, they are highly unlikely to result in disease. Proteins that do not exceed these criteria should present little or no risk of inducing CD.

2.9 Potential toxicity

Few proteins are toxic when consumed and most of those act acutely (e.g. ricin)[70]. The HOSU evaluation is a key consideration in addition to a bioinformatics comparison of the amino acid sequence of any newly expressed protein to the NCBI protein database using a keyword limit of "toxin" or "toxic". Although it seems there is a lack of published data on how to perform a bioinformatics evaluation for potential toxicity for a GMO, all GM products submitted to the US FDA or EPA must undergo an evaluation[71]. I have performed the bioinformatics searches for a few potential GM crops and novel food ingredients for regulatory submissions using the general NCBI protein database using BLASTP with keyword limits of toxin or toxic to focus on potential risks. Usually additional sequence comparisons are needed using the new protein in the search but without keyword limits to provide a relative comparison of other proteins with a known history of safe use or safe human exposure and the query protein (GM protein) or novel food ingredient. The process also requires a careful evaluation of published scientific literature related to the closest sequence matched proteins as well as the protein of interest. While bioinformaticians often claim that proteins sharing greater than 25% identity over their full-length are homologues and often have similar functions, most proteins with such relatively low identities do not share specific toxic properties or exact enzymatic functions. Therefore bioinformatics evaluations must be evaluated relative to other proteins. The results should guide decisions regarding a need for any toxicology testing, and if needed, the target organs and tests that might be useful to evaluate risks. So far there is no evidence that any protein introduced into a GM plant approved in the US has had a toxic effect of humans or other mammals.

In the US regulatory system, if a protein introduced into a GM crop is intended to have toxic activity to insects, bacteria, a fungus or have anti-viral activity, such as the plant incorporated pesticidal Bacillus thuringiensis crystal proteins, the proteins must be tested by an acute mouse toxicity test. The OECD guideline for acute toxicity testing (E425, 2001) is the model followed in many studies. The protein is gavaged into adult mice using a dose that is typically 1 000 fold higher on a mg protein per kg body weight, expected for human food consumption. Sometimes the excess dose is not quite so high, but normally at least 100 fold higher. The dose is given on day 0 and the health of the animals is monitored along with control (mock-dosed) animals for 14 days. At that time

body weights, blood samples appearances and clinical observations are collected. The animals are euthanized and gross pathology and if needed histology samples are examined for abnormalities. Usually there are 10 animals per sex per treatment group. Quite often two doses are used as separate treatment groups to ensure that any abnormality has a dose-effect. While there may be some statistically different findings for a few measurements between groups for the GM and control animals, historical weights and measures of the same strain of mice should be available for that specific toxicology facility to be able to evaluate unexpected differences. Some studies describing the acute mouse toxicity tests for approval of some GM products have been published[72-74]. In rare circumstances longer term toxicology studies are called for by regulators or critics of the technology, but the scientific justification for extra testing is usually quite weak. It is important to consider that unlike a number of organic compounds or heavy metals, consumed proteins do not accumulate in the body of mammals and toxic effects are expected to be acute rather than chronic.

Some countries (e.g. within the European Union) require an acute mouse test as well as a subchronic, 90-day whole-food feeding study in rats, or repeat dose testing with high doses of whole protein. While the 90-day study design is detailed in the OECD guidelines and a few published studies have been performed, there is not a good justification and little proof that such a study will identify known hazards[75]. The 90-day rat feeding study is more of a hybrid toxicology-nutritional study. Some regulators and critics suggest that the 90-day study provides a tool to evaluate "unintended effects" that might arise due to the insertion site of the new gene into the genome of the crop. It should be worth considering that the host (recipient) crops are normally species that have been consumed for centuries with good history of safety and that genetic variation in naturally bred varieties and lines have introduced many unintended genetic changes without introducing adverse toxic properties in the food.

Two studies designed to test the predictive value of the 90-day rat whole food feeding study using experimentally designed recombinant rice gave somewhat conflicting results[76-77]. The first tested a GM rice expressing the snow-drop lectin from Galanthus nivalis (GNA) and the authors conclude that the study failed to show the potential toxicity of the lectin. The second experimental GM expressed high levels of the common bean phytohemagglutinin lectin PHA-E, which did show toxicity when the protein was fed in raw form at high concentration. My interpretation is that the raw, uncooked PHA gave significant toxicity as would be expected to occur in humans consuming raw kidney or navy bean. The GNA study seems to have had negative results because the protein expression was too low in concentration or the protein was heated in feed preparation. Since humans can consume cooked kidney and navy beans, but not raw beans, it seems the test results were predictive of the human experience. It might have been more appropriate to test raw and cooked samples as two separate treatments. The assay is not very sensitive and there are severe limitations to the dose that can be feed compared to the human diet, typically much less than the 100 fold safety factor typically used in toxicity studies. Many toxicologists have questioned the usefulness of the 90-day whole food feeding study[9]. While others claim even more detailed, complex and expensive studies are needed to fully test potential toxicity[78]. However, a recent peer reviewed evaluation of published safety, toxicology and whole grain rat feeding studies on current GM crops provides objective evaluation of the overall approach and concludes that in most cases a 90-day feeding trial is not needed to evaluate safety, but results are certainly consistent with safety[79]. Interesting at a time when animal welfare groups and even the institutional animal care and use committees in many institutions are calling for reduced animal testing, some scientists involved in regulation or testing are calling for more unproven animal studies.

2.10 Additional toxicology studies

Questions should be asked about any new proposed toxicity test, as well as existing testing methods. What types of hazard can be or has been identified with a given test protocol? What is the rate of false positive and false negative results for each test? And finally, are there more effective tests that could be used? A number of recent publications have discussed the pros and cons of using alternative computer based, cell-based, or tissue based methods, primarily for pharmaceutical toxicology evaluation[80-81]. They focus on having a scientifically sound hypothesis, validated methods and historical control data as essential criteria. Understanding the limitations and benefits of the different models are essential in making a determination about tests that might be useful for evaluating potential toxicity. In most countries including the US, there is a general requirement by animal care and use committees to show that the specific test on the specific test material has not been performed previously unless there is a reason to doubt the results. Therefore repeating

the same animal tests on the same GM crop event in multiple countries is deemed unethical.

The final conclusion of toxicity evaluations should be either the GM crop does not pose any additional significant risk of toxicity compared to similar non-GM varieties, or that it does pose a substantial new risk. The FDA and EPA have been able to reach those conclusions for many new GM products if the developer followed the standard assessment process. Unfortunately some regulatory bodies (e.g. EFSA and the European Commission) in Europe and regulators in India and China continue to raise new questions about hypothetical concerns including potential adjuvanticity, alteration of fertility or the potential to induce cancers even though there are very few examples that any dietary protein could have such an effect. Those regulators then fail to approve products for which there is no evidence of risk. The US regulatory agencies have emphasized the need to use proven methods to evaluate safety of novel proteins and GM products. They have not asked (so far) for additional studies that are not already demonstrated to help assess safety. However, if a developer provides data from a new evaluation, they will consider it, although it may delay approvals or acceptance.

3 Evaluting GM products for unintended effects

The methods and genetic modifications used to generate the herbicide tolerant or insect protected traits that have been widely adopted following regulatory approvals introduce relatively minor variations in the host plant genomes compared to those introduced through "natural processes" of mutations and reproduction. Interestingly those "unknown" natural changes are not characterized except by phenotypic variation and they have evolved to provide the diverse genetic background needed to allow plant survival with challenges of plant diseases and pests, and diversity of climactic conditions and soils. The GMOs on the other hand have been characterized in insertion site, copy number, gene sequence and encoded products. If the introduced gene encoded an enzyme, metabolites of the enzyme would have to be evaluated. Interestingly, a good portion of the maize (Zea mays) genome is made and modified by transposons that were described as "jumping genes" by Barbara McClintock from her studies in 1948. She was awarded the Nobel Prize in Physiology in 1983 for that discovery[82]. A recent study identifying genes in 503 genetically diverse lines of maize found ~ 16% of the genes are not present in all 503 lines, showing marked genetic variation[83]. The bread wheat we consume today (Triticum aestivum) is encoded by three sets of chromosomes (thus is an evolutionary hexaploid) of relatively primitive grass species so that most proteins in wheat are encoded by three sets of divergent genes that are nearly identical in some cases, or very different. In addition, the replication of genomes through sexual reproduction allows gain or loss of function and extension of the capacity of the plant to grow in different environments or have multiple options for nutrients (or anti-nutrients). Bread wheat and pasta wheat (Triticum durum or Triticum turgidum subsp. durum, an evolutionary tetraploid) are both nutritious and used extensively in human food. But both cause celiac disease in about 1% of the general population in North America and Europe, genetically susceptible individuals (25% of the population) and IgE mediated food allergy in a much smaller number of people (<0.4% of the public). Those are non-GM crops as there are no approved GM wheat varieties (yet). That illustrates that all foods represent some risks for some consumers and that it is necessary to have genetic variation to produce the foods we eat.

We should step back and consider why we eat certain foods like rice, wheat, soybeans and maize and other foods, but as humans we do not eat grain alone. Humans have selected certain food sources for ease of production but mostly for nutritional value, measured by average energy, amino acid composition, lipid content, carbohydrates, vitamins and minerals. Those crops were initially grown and consumed long ago and dramatically changed by breeding and cultivation without any scientific measure of specific amino acids, caloric density, vitamins or fatty acid profiles. In the past 100 years we have learned how to measure those components and also in many cases believe we know what a "healthy" and "nutritious" diet is made up of. Typically it is a mixture of different foods. Even though we have all that information today we do not make detailed measurements of the composition of every shipment of grain that goes into a box of cereal or a loaf of bread because would cost too much and we also know that on the average safety and nutrition of the cereal or bread is fine. We have learned the primary components of each major food crop and have typical measurements that are tested by agricultural nutritionists to ensure they formulate optimal diets for agriculturally important species. Each crop has specific components that are evaluated, and nutritionists have ranges that they deem acceptable for animal feed.

3.1 Key nutrients and anti-nutrients

Key nutrients and anti-nutrients expressed in the host plant (gene recipient) are to be measured and evaluated relative to non-GM varieties or lines intended for the same uses. There is an expectation that the key components will fall within the range the same components in non-GM events of similar genetic background[84-85]. But as with all statistical measures, statistically significantly different values are expected when measuring many components. However, statistical differences alone are not a reason to reject a product as unsafe; there should be a scientifically based rationale to suggest potential harm. In order to provide guidance on appropriate compositional traits for given food crops recent historical records for varieties of the same crop must be found or a number of commercial varieties must be planted in adjacent plots in multiple field trials.

Animal nutritionists understand the differences in compositional measurements that are important for canola, corn, cotton, potatoes, soybeans and wheat. And many possible compositional measurements are irrelevant to the typical use of these crops. However, some GMO regulators and critics expect that developers will measure every possible component of the GMO and compare it to the nearest genetic relative. If there are statistically significant differences some would argue it is due to the insertion of the DNA and that the food is unsafe. Yet we have also learned that plants from genetically identical plants grown in close proximity or 100 miles apart can differ in many components due to micro-environmental differences. The complexity of the genotype and environmental interactions that can lead to significant differences in expression of some components of agriculturally important crops has not been sufficiently evaluated in terms of biological relevance, yet some scientists are calling for increasing the use of various omics-techniques to measure variation with high precision (Doerrer et al., 2010). Fortunately, even though the compositional analysis is considered an important part of the safety assessment of a GM crop, in the US and most countries regulators have not blocked an approval of a GM food or feed crop due to minor statistical variations in composition as it is clear that non-GM products often have fairly marked differences in components without measurable effects on food or feed safety (Privalle et al., 2013). An important recent finding by two different groups is that compositional differences between GM and near-isogenic lines are primarily due to back-crosses and conventional breeding and are not caused by insertion of the gene[86-87]. Understanding the source of variation is an essential consideration as some authors are suggesting complex proteomics analysis of potential differences in endogenous allergen levels in GM plants might be due to insertion and require additional tests[88].

Thus we need further definition of the important components to measure and guidance on the variation of those components that may have biological relevance. In order to provide some references for composition, the biotechnology industry supports the International Life Sciences Institute (ILSI) Crop Composition Database[89] that contains compositional data for seed of corn, cotton and soybeans (https://www.cropcomposition.org/query/index.html). The data is limited to years 1995-2005 and specific countries of cultivation. The ILSI database is scheduled to a new version released by the end of 2014 that will include many more data-points and expand to include sweet corn, canola and rice. Additional information is available for rice and soybeans from a Japanese composition database (http://afdb.dc.affrc.go.jp/afdb/index_e.asp). The data is available for a limited set of varieties of these two crops and limited years of cultivation from Japan[90]. These databases provide some information about methods and ranges of components specific for the species. Interestingly the animal feed industry is most sensitive to changes in composition of commodity crops as slight variation in feed quality can mean profit or loss to major animal producers. Companies like Tyson (USA), with more than 4 000 poultry farms in chicken production and Perdue Farms (USA), second leading poultry producer in the US measure composition of feed based on nutritionally important ingredients that are crop-specific. In order to formulate optimum feed for growth and safety they measure proximate analysis of every delivery of commodity crop getting random representative samples from their extremely large shipments, measuring total protein, lipid, carbohydrates, moisture, ash, fiber and often the amino acid composition as well as crop specific vitamins, fatty acids and minerals. They also measure crop specific toxicants and anti-nutrients. The poultry industry is the most sensitive to nutritional quality changes. In addition, every shipment of corn grain or dry distiller's grain is checked for mycotoxin levels as corn is the most likely crop to have contamination. An example of a chicken broiler study on a GM stacked-trait event describes the feed ingredient evaluations and provides real data that would be similar to the analysis performed

by Tyson or Perdue[91]. The major components and measures essential to optimum chicken growth that are evaluated in feed preparation do not include a long list of metabolites, RNA transcripts or proteomic measures. Instead they focus on components that are known to contribute to the substantial growth rate (approximately a 35-fold increase in body weight from hatching through day 42 of the studies) for the chickens. The feed efficiency and weight gain are highly correlated to nutritional properties, more so than any other animal species. The production of feed lot size and the number of animals in commercial production units is normally quite large. Since chickens are fed defatted soybean meal, the composition of fatty acids and lipids is not as critical for soybean ingredients as it is for mammalian species, such as dairy cows. Most dairy farms, beef, pork, goat and sheep operations do not monitor every shipment of feed, except for mycotoxins in corn, but instead sample occasionally throughout the year to re-formulate diets if the typical component nutritional values are changing. In the US studies supplied to regulatory agencies include proximate and specific ingredient measures comparing the new GM line ingredients (seeds, grain or forage) and ingredients from a nearest genetic comparator of non-GM line and ingredients from three to five other commercial non-GM lines, all grown at multiple geographical sites to provide environmental diversity for plant growth. Some countries But in general a GMO developer must provide specific composition to regulators from multiple years of multiple geographical replicates of the GMO and a number of non-GM comparators to allow statistical comparison. The relevance for safety is usually not clear.

In addition to nutrients, specific anti-nutrients are also measured that are crop specific, including lectins and trypsin inhibitors, toxins such as solanine and allergens for highly allergenic crops (e.g. soybean). While there are generally accepted limits for many anti-nutrients (e.g. solanine at 200 mg per kg fresh weight, Friedman, 2006), acceptable limits of variation allergens have not be established[92].

3.2 Measuring potential changes in endogenous allergen levels

There is a requirement in the US and a recommendation by the EU to consider whether insertion of the transgene has increased the expression or accumulation of naturally occurring endogenous allergens if the gene recipient (host plant) is a common source of food allergy. Regulators recognized that the risk of food allergy is not equal from different allergenic sources. Labeling requirements for processed foods are meant to be truthful and to protect those at risk. In the US and in the EU food labeling regulations demand that all ingredients derived from the major allergenic sources must be labeled. That list includes the eight common allergenic food in the US: chicken eggs, cow's milk, peanut, many tree nuts, crustacean shellfish, fish, soybeans, wheat (http://www.fda.gov/food/resourcesforyou/consumers/ucm079311.htm). In addition in the US foods containing glutens from wheat, barley and rye must be labeled, unless the gluten content is less than 20 ppm on a mass basis. In the EU six more foods are added to the list of eight including: cereals containing gluten (wheat, rye, barley, oats, spelt, kamut and hybrids of those grains); celery (root), mustard seed, sesame seed, lupin and molluscs as well as sulphur dioxides) as be listed all whole, relatively unfractionated ingredients must be labeled as to source (e.g. wheat, eggs, milk). In both the US and EU ingredients derived from the commonly allergenic foods must also be labeled unless the processed ingredient is exempt (e.g. hexane refined soybean oil). Starch from wheat must be labeled as coming from wheat, but starch from corn, rice or tapioca may simply be labeled as modified starch, without indicating the source. Thus in the US and EU a developer must evaluate potential changes in endogenous allergens in GM peanuts, soybeans and wheat, but not in common beans (*Phaseolus vulgaris*), corn and rice as they are not common sources of allergy. The methods used to perform the evaluation have generally been consistent with measurements of allergens in diagnostic allergen products[93-94]. Pharmaceutical grade allergen extracts are expected to show similar qualitative binding using immunoblots as well as variation in total IgE binding between 50% and 150% of the extract standard mean serum IgE binding using pooled allergic sera to compare one batch of allergen extract to a previous batch[93,95]. The first herbicide tolerant soybean (event 40-3-2 from Monsanto) was tested for differences in IgE binding using western blots of soybean extracts separated on SDS-PAGE with sera from three individual soybean allergic subjects[96]. Sten et al.[97] performed a much more extensive, non-regulatory study of IgE binding by in vitro methods using sera from 10 soybean sensitized subjects to compare results between 10 genetic varieties of the same GM event (40-3-2) and 8 genetically similar non-GM varieties of soybean. They used RAST-inhibition and basophil histamine release and found no significant difference between the GM and non-GM soybeans although there

were marked individual subject to subject and soybean line to soybean line differences. My laboratory has also performed serum IgE binding studies on five different soybean events in total from three different commercial developers. The methods used included direct IgE binding, ELISA inhibition with pooled soybean allergic sera or direct ELISA with individual sera and found no significant differences in binding except between one or more of the non-GM lines[92,94]. Some differences were found in gain or loss of an IgE binding band in the qualitative IgE immunoblots in some non-GM soybeans. In addition to those standard methods for evaluating potential changes in allergen abundance, two-dimensional (2D) immunoblots were performed using individual sera to compare each GM to three non-GM soybean lines due to new regulatory demands by the EFSA[51] and EC regulations[52]. Some individual serum IgE binding spot differences were noted, but not showing specific changes for the GM lines[92,94]. Clearly the population of allergic subjects included in such studies will influence the outcome. It is impractical to include more than a few (10?) specifically allergic subjects in a study unless multiple large allergy centers are included. There will always be some uncertainties regarding which proteins and isoforms might bind IgE from individual allergic subjects. However, the suggestion by the EFSA to use proteomics (LC-MSMS) to evaluate the abundance of individual "allergens" in soybeans and other commonly allergenic food crops is not as valid as serum testing because the list of "allergens" that EFSA wants to use [e.g. allergenic proteins in the OECD composition list for soybeans, includes proteins with little or no evidence of allergenicity (Gly m 1, Gly m 2, Gly m 3 (profilin), P34 Gly m Bd 30 K, Unknown Asn-linked glycoprotein, lectin, lipoxygenase, Kunitze trypsin inhibitor, unknown 39 and 50 kD proteins and[22-25]. The important allergens in soybean that have been identified include Gly m 5 (β-conglycinins alpha-, alpha'- and beta-) and Gly m 6 (5-glycinins) and possibly Gly m 4, also known as SAM22. Thus the EFSA recommendation is not based on evidence of risk since there is no gradation of risk in the proteins chosen and in fact some have no published evidence of allergy, or the protein sequence was not determined. In addition, LC-MSMS does not provide 100% coverage of any protein and it is therefore unlikely to identify isoforms, some of which may not bind IgE. Serum IgE binding tests at least compares a biological measurement between the GM and other non-GM varieties using sera from allergic subjects.

However, it is important to consider whether there is relevance for safety to these measurements. Is there an increased risk of allergy if there is a difference? People allergic to soybean should avoid eating any soybean. People who are not allergic can eat as much as they desire. In processed foods the amount of total soybean protein can vary markedly from product to product and the food companies are not choosing lots of soybean based on specific varieties. Instead they buy in bulk with the soybeans typically mixed at the silo, during shipment, in milling and processing and during food manufacture.

An important question that has not been answered by any scientific study or any regulatory body is what difference would be required in endogenous allergen accumulation to have an adverse impact on human health for the specifically food allergic subjects who are the sensitive, at-risk population? An informative estimate might be made based on the dose-increase interval highly trained clinical food allergists use in performing double-blind placebo-controlled food challenges (DBPCFC). There are a few publications describing protocols for testing high risk patients with the intent of establishing thresholds of doses for various allergens. A review of studies by Crevel et al.[98] reported protocols with increasing challenge doses between 3-fold and 10-fold for peanuts with peanut allergic subjects. The experimental design for DBPCFC in the EuroPrevall studies began at three micrograms of protein from allergenic sources and used ten-fold increasing doses to 30 mg of protein, then reducing the step increase to three-fold above 30 mg as the risk of serious reactions were felt to increase above that dose[99]. Therefore it seems logical to conclude that at least a three-fold increase might of concern.

3.3 Assessing potential new, unintended proteins

During characterization of each new GM event the insertion of DNA is to be analyzed to confirm the sequence of the insert as well as the immediate surrounding DNA. Typically a few hundred bases to a thousand bases are provided beyond the insert. The sequence of the insert is to ensure that the protein(s) intended to be expressed (if any) are correct. If an unexpected change has occurred, that should be evaluated in terms of the function of the new protein as well as possible risks for allergy and toxicity using bioinformatics. The flanking DNA is considered to determine if there is a possibility a new fusion protein might be expressed in the plant. All six potential reading frames in the DNA sequence are evaluated using computer algorithms to identify potential open

reading frames (ORF). Some regulators are satisfied with start (methionine) to stop codons to define a potential ORF. Others want all hypothetical ORFs meaning stop to stop. The potential ORFs are then evaluated using bioinformatics to search for matches to allergens and toxins. The critical segment is the fusion site. The plant DNA on each side of the insert was already there and if it encoded an allergen or toxin, those would have been endogenous hazards. The safety assessment is focused on new potential hazards and risks. If there were matches to an allergen or a toxin, further analysis may be performed to evaluate whether and what tissues would transcribe RNA from that region of the DNA. If the specific RNA is present, measurements could be made to determine if there is translation product (protein) using either LC-MSMS or antibodies generated against a synthetic peptide "encoded" by the ORF in assays. If there is a negligible level of protein, then the risk is minimal.

Some regulators ask for flanking sequence until it is clear that the transgene has not interrupted an endogenous plant gene in the coding or intervening sequences (introns). However evaluation of agronomic traits of the plants in field trials with geographical replicates will help identify any biologically significant differences of the GM vs non-GM varieties. That type of evaluation is about performance of the plant, not safety. The US regulators very interested in obtaining information relevant to safety of the food and feed products. The GM developer and associated seed companies must show data to farmers to convince them that the GM plants produce adequately in terms of yield and overall composition. Otherwise farmers will not purchase the seeds.

3.4 Assessing unintended effects conclusions

The conclusion of the compositional analysis is generally whether the total nutrients and anti-nutrients for the specific crop are substantially equivalent to non-GM comparators or not. These analyses are performed using field-trial grown material of the GM and non-GM varieties in geographical replicates. Certainly there can be some statistically significant differences of measuring a number of components in many samples over different geographies will often result in a few statistically significant differences. Most of the variation is due to actual genetic differences that are associated with the whole plant genomes and back-crossing and breeding programs and have nothing to do with transgene insertion[82-83]. In addition, recent discoveries that DNA methylation patterns can be inherited and alter gene expression without any change in DNA help us realize that we cannot expect to control or understand every measureable difference based on DNA sequence information[100]. And it is extremely important that we realize that every measurable difference does not constitute a risk for consumers, in fact very few do. Humans selected and have improved most of the domesticated crops hundreds to thousands of years ago. We know that genetic variation is needed to be able to grow the same species in a wide variety of environmental conditions in order to produce food and feed.

In the US the values from individual measurements are compared between the GM event and the near genetic relative (near isoline or parental variety) and also compared to either a number of commercial lines grown in the same field trials or recent historical data from real production samples. If the measures from the GM crop fall within the typical range of variation as a benchmark for potentially relevant biological differences, a difference between the GM and near genetic relative is considered acceptable. The GM plant is therefore deemed "substantially equivalent" to other varieties of the crop. Similar inferences are made from data obtained by measuring animal responses in feeding studies such as the 90-day rat feeding trial that some regulators require; 42 day broiler studies or large animal feeding trials that are generally used as industry acceptance studies in many countries, but are required in some (e.g. India).

3.5 Current status of GMO approvals

How many GM events have been developed and gotten regulatory approvals for growing, of use as food and feed? It is hard to find accurate information. The Center for Environmental Risk Assessment (CERA) GM crop database www.cera-gmc.org/GmCropDatabase lists 153 total crop-events. Not all of those were developed through GM technology as some were developed by mutagenesis or traditional breeding. In addition, not all of those are approved anywhere and some are approved but not used. The International Service for the Acquisition of Agri-Biotech Applications (ISAAA) also maintains a GM crop database that lists 353 events (www.isaaa.org/gmapprovaldatabase). By quick examination it seems ISAAA shows some crop types not listed by CERA including beans (*Phaseolus vulgaris*), eggplant or brinjal (*Solanum melongea*), poplar trees (*Populus* sp.), sugar cane (*Saccharum* sp.) and pepper (*Capsicum annuum*) that have not been submitted to U.S. or Canadian regulators. It is likely that each of these databases misses a few events, but unlikely that either

of them miss globally traded GM crops. In addition the three US regulatory bodies each have a separate database that presents their actions on individual GM events. The USDA website is: http://www.aphis.usda.gov/biotechnology/petitions_table_pending.shtml. The FDA website is: http://www.accessdata.fda.gov/scripts/fdcc/?set=Biocon. The EPA website is: http://www.epa.gov/oppbppd1/biopesticides/pips/pip_list.htm.

Even though many events with different properties and in different plants are approved for use, the bulk of the GM events are in a few commodity crops (canola, cotton, maize, and soybean). The rate of adoption as measured in percent of hectares planted in GM crops in the U.S. has been extremely rapid, going from zero in 1994 to more than 90% of our soybeans and corn (maize) in 2014. A significant fraction of the cotton production in the U.S. is from GM events while 95% of cotton in India and 90% of cotton in China is GM cotton. There are now multiple events from different developers having similar functions (herbicide tolerance or specific insect resistance). At the same time a number of previously approved GM crops (post-1994) have disappeared from the market. Some products were dropped due to consumer or company pressures including the viral resistant, Colorado potato beetle resistant potatoes developed by Monsanto as major potato markets are dominated by French fry and fast food restaurants that are very sensitive to perceived consumer preferences. Those products that dramatically reduced insecticide use on potatoes were withdrawn in about 2002 due to pressure from the fast food industry. Herbicide tolerant wheat was submitted by Monsanto to Canada and the US, but was withdrawn before approval due to pressure from the Canadian Wheat Board because of fears export markets to Asia would block trade. Delayed ripening tomatoes were dropped as they were not commercially viable (four companies including Zeneca and Monsanto had approved GM events) because fresh food qualities were not as good as non-GM varieties. The viral resistant squash that was developed by Asgrow is still on the market, though now owned by Seminis. Viral resistant papaya was developed by researchers at Cornell University and was approved for use in the US because the Hawaiian trees were being decimated by ring spot virus. The GM construct blocked replication of the virus and the introduction of this trait saved the industry in Hawaii.

4 Summary

Some experts predict an eminent global food crisis while others suggest continuation of more regionalized crises that may be caused by local draught, disease or have artificial political or economic causes[101-102] (Butler, 2009a; Butler, 2009b). Some solutions for improving the global sustainable agriculture are and can be contributed through biotechnology, with current and future GM crops. Yet progress is being stifled by a very focused, well financed vocal minority of NGOs and by celebrities who are stirring public uncertainty even though they clearly do not have a good understanding of agriculture, food production and costs. Can we find common ground in this debate? Few who are students of food production, agriculture and human health would deny that at some point the world's growing global population will outstrip the capacity to maintain food production in in the long-run even though the efficiency of production has increased markedly in the past century[103]. Yet our ability to increase production currently comes through the use of adding mined minerals, increased use of fossil fuels for fertilizer and tillage and the use of machines to replace human labor and draft animals in intensive agricultural practices. Can we maintain our current rate of expansion? Experiences in the US agricultural system may provide useful examples for the potential benefit of GM crops in China and other Asian countries and in setting a standard for food safety of newly developed products.

In considering the experiences in the US regarding the safety evaluation process and regulations of genetically modified (GM) crops, it is necessary to look also at the global nature of food supplies, the concerns of various food safety regulatory bodies as well as consideration of the long history of various food crops. No country is self-sufficient and most foods consumed in any one country originally came from, or is dependent to some extent on inputs from other countries. The adaptation of wheat, rice, potatoes, tomatoes, peppers, various legumes and the specific animals we consume today were developed from naturally occurring ancestral organisms from very different geographical locations than those used for production today. They were selected and improved through breeding processes that took hundreds or thousands of years. They were chosen by experience, but based on food utility (nutritional and anti-nutritional) characteristics, ease of production and food safety. Yes there are real risks of foods for those with allergies and celiac disease. There are risks for those who do not prepare or store food properly to suppress microbes and spoilage and to inactivate anti-

nutrients. The primary potential risks of new proteins are relatively easy to prevent through the current assessment scheme.

There are a few uncertainties that US regulators are still finding perplexing. Primarily if the protein is stable in the pepsin assay, they are concerned that it might sensitize and become an allergen. Low abundance stable proteins have little risk, and they should find an acceptance level. We also need to continue working on a better way of predicting sensitization. The current suggestions of computer programs to predict antigenicity are far from perfect and over-predict risk. Animal models so far have failed to provide sufficiently accurate predictions to be useful. Cell based assays using human antigen presenting cells, T cells and B cells have not been validated to demonstrate accurate predictions. Therefore additional research is needed for difficult proteins where the current Codex guideline[1] and US evaluation process do not show results leading to a conclusion of unlikely harm. But most GM products today are easily cleared with bioinformatics for allergenicity, celiac disease and toxicity. In a few cases serum IgE tests are needed and simple, predictive toxicity tests are needed.

Labeling of foods is a major obstacle around the world. Some countries like China have rules demanding labeling at least some foods if they contain GMOs. In the US a few states have passed laws that may take effect in the near future and a few states will vote on labeling in November, 2014. Major economic and practical food production hurdles make this approach untenable. Crops are grown and traded across state lines and national boundaries. Food companies often make products for all 50 states and for export. There are many individual ingredients that might contain a GMO, but that is not consistent from lot to lot. As an example, figure 2 shows the labeled ingredients in a black vegetarian bean burger produced in the US. Each component derived from soybeans, corn (maize), canola or cotton may be from a GMO. The CERA GM crop database (http://cera-gmc.org/index.php/GMCropDatabase) lists 12 approved GM soybeans representing 8 GM proteins and 57 approved maize lines representing at least 15 different proteins. Suppliers of commodities, ingredients and final food products would have to control and test for all of those ingredients if they do not want to list "GMO" on the label if these laws pass. There will be added expense,

Ingredients: Black Bean Veggie Burgers

(frozen meat-substitute meal, by a US company)

WATER, COOKED BLACK BEANS (BLACK BEANS, WATER), COOKED BROWN RICE (WATER, BROWN RICE), ONION, **WHOLE KERNEL CORN**, **CORN OIL**, **SOY PROTEIN CONCENTRATE**, WHEAT GLUTEN, EGG WHITES, DICED TOMATOES, BULGUR WHEAT, GREEN CHILES, CALCIUM CASEINATE, **CORNSTARCH**, CONTAINS TWO PERCENT OR LESS OF ONION POWDER, SPICES, TOMATO JUICE, YEAST EXTRACT, TOMATO POWDER, DEXTROSE, SALT, GARLIC POWDER, HYDROLYZED VEGETABLE PROTEIN (**CORN GLUTEN**, WHEAT GLUTEN, **SOY PROTEIN**), **SOY SAUCE** (***SOYBEANS***, WHEAT, SALT), NATURAL AND ARTIFICIAL FLAVORS, PAPRIKA, JALAPENO PEPPER, CITRIC ACID, XANTHAN GUM, DISODIUM INOSINATE, , CARAMEL COLOR, LACTIC ACID.

Allergen Information:

CONTAINS: **SOY**, WHEAT, EGG AND MILK INGREDIENTS.

Fig. 2 Ingredient label of a commercial black bean burger produced in the US in 2014. Ingredients that may contain a currently approved GMO are listed in bold and underlined. Those ingredients may be subject to GMO labeling laws if mandatory labeling laws are passed. The Allergen information is a safety label as it shows major allergenic ingredients that have to be avoided by some consumers with specific food allergies so they would know to avoid this product for safety reasons if they are allergic to soybean, wheat, eggs or milk.

and no safety benefit. For foods that are already cluttered with labeled information, critical safety information such as allergen content gets lost.

The foods humans consume are tremendously diverse in composition, nutritional qualities and to some extent, risks. We are omnivores and our ancestors adapted to many different climates and conditions as they spread across continents and changed from migratory hunter-gatherers to migratory pastoralists and then to relatively sedentary agriculturalists[104,106]. The adaptations seem to have been possible because of the ability of humans to cooperate and accept added costs of helping to ensure survival of others rather than protection of the immediate family, an adaptation that was not always beneficial to the immediate relatives, but was beneficial for the society[106]. In the post-industrial era humans have become highly mobile individually. However, within each society the basic food production infrastructure needed to maintain the population is slow to change for many reasons including the large investment in equipment, complexity of the commodity and food processing facilities and the relatively restricted genetic pool of plants and animals that are used for production. But adaptation occurs and the efficiency of production has increased, especially during the last century. Increased have occurred even as land is available for farming as the population concentrated in cities away from the production of food crops[107]. Since the world population is now estimated to be over 7.25 billion people, and with a total biomass exceeding the combined total of all other terrestrial vertebrates we need to think hard about how to improve food and feed production. It took hundreds or thousands of years to learn how to manage and accept many new methods of food production. In the past 100 years food production has shifted markedly to more industrialized methods to meet food demands. Some people would seek to stop the technology, restrict the tools of introducing new improvements into food crops because of claims they produce unsafe foods. But as I search for evidence of harm from GM crops, it is not there.

It is helpful to consider that none of the plant foods that we grow and consume today are completely natural. Although they are genetically fairly similar to some native plants, the grains (wheat, barley, rye, rice, maize, sorghum) have been bred and selected for hundreds of years. Many varieties of tomatoes, potatoes, eggplant and peppers are quite safe for consumption after many forms of cooking and processing. But they are closely related, in the same plant family (Solanaceae) as toxic nightshade, which along with tobacco and petunias are really not edible. The edible solanaceous plants have wild relatives in the same species that produce sufficient levels of glycoalkaloids (solanine, tomatine and others) and lectins that are quite harmful to us and to many domestic animals if consumed. We can only consume the current varieties of these crops because our ancestors went through a process of breeding and selecting varieties with low levels of these toxins and anti-nutrients in the edible plant parts. They did that without the complex scientific tests and instruments we use today to detect specific substances that cause harm. They did that without having standardized animal feeding trials. Even though we are omnivores and can consume many different plants and animals, we have had to learn the limits of what we can consume. And even though the potatoes that we eat today are safe, we have learned that some wild relatives produce sufficiently high concentrations of a solanine, tomatine and other glycoalkaloids to cause harm or even death.

Beyond a historical perspective, it is also important to remember that we live in an age of increasing information distribution with frequent unintended impacts of miss-information. There are many claims of real or potential harm from various foods that would never have been noticed centuries or event decades ago, but often the communicated fears are hypothetical risks. However, instant messaging and the internet compress years to seconds. When European explorers brought tomatoes and potatoes from South America to Italy and the United Kingdom in the 1500's they were introducing crops that had been grown and consumed safely for over a thousand years. But in Europe people did not have full knowledge of how to grow and use the plants. Some who became ill due to improper food preparation or eating the green part of the plants and after falling ill people suggested that the entire plants were poisonous including the fruits and tubers. Natives of South American knew to avoid consuming the green plant material. The rare cases of harm in Europe lead to wide spread fear that stifled the introduction of these now staple foods into the European diets. Now false claims about GMOs are common and effects long lasting. Recent claims by Dr. Oz, Jeffrey Smith, Oprah Winfrey or Cui Yongyuan claim that GM crops are unsafe or untested have caused consumers to become skeptical of claims by biotechnology companies and governments that they are safe. Those media personalities however have not read the dossiers or performed safety studies that have convinced US regulators the products like European Corn Borer

resistant MON810 is safe. How do we present the truth to consumers when there are "trusted" personalities telling consumers that the government is corrupt and that big biotech companies like Monsanto did not do an adequate job of testing and evaluating safety?

5 Conclusions

The US regulatory system for evaluating the safety of GM crops involves three federal agencies, the USDA, the FDA and EPA. The process for evaluation was initiated in the late 1980s and early 1990s through consultations that included academics, industry scientists and governmental regulatory scientists and policy makers. The assessment was refined in the late 1990s through 2003 and aligned with the Codex Alimentarius Commission Guidelines for the safety assessment of GM crops. Potential risks of allergenicity of foods produced from the GM crops must be evaluated using scientifically acceptable methods. The process is efficient for identifying proteins that are likely to present a significant risk of food allergy, which would be the transfer of a known allergen or a likely cross-reactive protein. There is a bit less certainty trying to predict whether a new protein with no obvious risks factors might sensitize de novo, but risks are clearly low in those cases where the protein is rapidly digested by pepsin in a test-tube assay and/or low in abundance in the food component. The potential that a transgenic has significantly higher expression of endogenous allergens is quite low compared to non-GM varieties, but in addition the risk is for those consumers who should be avoiding consumption of food from the host plant anyway. Thus there is no practical increase in risk even if the content of endogenous allergens was increased. Potential food toxicity is also evaluated based on criteria established for non-GMOs. Few proteins are toxic and the comparison of the sequence of the GM protein to those of known toxins along with evaluation of the gene source and the mechanism of action of the protein will identify high risk proteins. The US has evaluated and approved the commercialization of approximately 100 new events or varieties of GMO. There are no documented cases where an approved GM crop has caused harm to humans or animals who have consumed edible parts of the plants. However, the regulatory process is expensive and time consuming. Since most food crops are traded on an international market, it is unfortunate that there isn't a single safety evaluation process that is standardized and accepted across all countries to avoid duplication of studies.

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