資料 5

鷲谷委員要求資料
(米国における **Bt** 作物の栽培基準について)

1．米国におけるBt作物の栽培基準についての概要

2． Bt ワタの栽培基準

3． Bt トウモロコシの栽培基準

4． Bt トウモロコシの栽培様式の模式図

5． Biopesticides Registration Action Document: *Bacillus thuringiensis* (*Bt*) Plant-Incorporated Protectants (EPA October 15, 2001)の抜粋(英文)

http://www.epa.gov/oppbppd1/biopesticides/pips/bt_brad2/1-overview.pdf

http://www.epa.gov/oppbppd1/biopesticides/pips/bt_brad2/4-irm.pdf

1　米国における **Bt** 作物の栽培基準についての概要

「生物農薬登録に係る行動文書」のうち、「*Bacillus thuringiensis* (*Bt*) 植物において発現する殺虫成分」について｛Biopesticides Registration Action Document: *Bacillus thuringiensis* (*Bt*) Plant-Incorporated Protectants (EPA October 15, 2001)｝から抜粋し、要約

(1) **Insect Resistance Management（IRM）Program** による **Bt** 耐性害虫の発生抑制対策について

無制限に Bt 作物を栽培すれば、標的害虫に Bt に対する耐性を発生させてしまうことが懸念される。Bt 耐性害虫を生み出してしまうことは複数の Bt 作物の有益性を損なうばかりか、微生物農薬である Bt 剤の効果も失わせてしまう。Bt は効果的な殺虫剤であるため、その効力が失われれば毒性の強い殺虫剤の使用へシフトするだけでなく、有機栽培農家の有効なツールも奪い、農家には経済的にも損失が大きくなると考えられる。そこで、米国環境保護庁（EPA）は Bt 耐性害虫の発生抑制対策として、Bt 作物の登録者に対して Insect resistance management (IRM) program の履行を義務づけている。

IRM プログラムでは、

① Bt 作物の栽培面積に関わらず契約上の義務として、隣接して非 Bt 作物栽培区（refuge）を設置させること、
② Bt 作物を購入するすべての利用者(農家)に対し「契約条項として IRM プログラムに従い、2003 年まで年 1 回契約履行義務を確認する」とした内容の利用者同意書にサインさせること、
③ Bt 作物利用者(農家)への IRM に関する教育プログラムの実施、
④ IRM プログラムの履行に関する追跡調査、
⑤標的害虫の Bt 感受性のモニタリング、
⑥耐性害虫が発生した場合の回復行動プランの履行、
⑦年1回の IRM 等に関する活動報告

を要求し、Bt 作物利用者が IRM プログラムに従わなかった場合、Bt 作物を購入する権利を失うことに責任を負うものとしている。

(2) **high dose / refuge strategy** の考え方

Bt 耐性害虫の発生抑制の具体的な対策としては「high dose / refuge strategy」の考え方に基づき IRM プログラムの中で実行されている。「high dose / refuge strategy」とは、Bt 作物栽培区では Bt 作物内で Bt toxin を高発現させること（high dose）で可能な限り標的害虫を駆除する一方、Bt 作物栽培区に隣接して(あるいはその一部に)十分なサイズの非 Bt 作物栽培区（refuge）を設けて Bt 感受性個体を繁殖させ、Bt 耐性害虫と感受性害虫を交尾させることにより、当該害虫集団内における耐性害虫の発達を遅らせる方法である。

この「high dose/refuge strategy」では、

① Bt toxin に対する抵抗性は遺伝的に劣性であり、抵抗性は対立遺伝子の単一遺伝子座によって与えられている。すなわち遺伝子型は感受性ホモ（SS）、感受性ヘテロ（RS）および抵抗性の劣性ホモ（RR）である、

②抵抗性の対立遺伝子頻度は低く、ごく少数の抵抗性個体（RR）だけが Bt 作物を摂食しても生き残る、

③ Bt 作物上で選抜された抵抗性個体（RR）は、非 Bt 作物栽培区で繁殖した感受性個体とランダムに交尾する、

④抵抗性の対立遺伝子に関してヘテロな個体（RS）は、Bt 作物内で発現している Bt toxin によってすべて駆除される

ことが前提条件となり、Bt 作物栽培区では RR だけが生き残るが、相対的な数としては SS が多く、両者が交配した RS は Bt 作物を摂食することで死滅することから、R の対立遺伝子は一掃され、抵抗性の獲得が遅れる、といった考え方である。

(3) Bt 耐性害虫の発達予測について

IRM プログラムについて害虫に耐性が発達するのに要する時間的な予測モデルによれば、飼料用 Bt トウモロコシによる Bt 量が標的害虫を殺虫するのに十分 high dose であるなら、コーンベルト地帯で 20 %の refuge が設けられている限り、トウモロコシの主要害虫である European corn borer（アワノメイガ）の場合は少なくとも 99 年間は Bt 耐性は進化しない。また、ワタの栽培地域で飼料用 Bt トウモロコシを栽培する場合は、50 %の refuge が設けられている限り、トウモロコシおよびワタの主要害虫である Corn earworm（アメリカタバコガ）では少なくとも農薬登録期間中は Bt 耐性を遅らせることができると予測されている。

（参考文献）

1) Biopesticides Registration Action Document: *Bacillus thuringiensis* (*Bt*) Plant-Incorporated Protectants (EPA October 15, 2001)

2) Mendelsohn, M. et al., Nature Biotech, 21 (9): 1003-1009 (2003)

3) Andow, D. A., Assessing Environmental and Human Health Effects.: pp. 99-124 (2002)

4) 白井洋一「害虫抵抗性遺伝子組換え作物が非標的昆虫に及ぼす影響：現在までの研究事例」　応動昆　47 (1):1-11 (2003)

2　米国における **Bt** ワタ（チョウ目害虫に対する **Cry 1Ac**）の栽培基準

Bt ワタの refuge については Bt 作物栽培者が次の3つのオプションから選択する。

オプション1：ほ場外における殺虫剤無散布の refuge 設定

① Bt ワタの栽培地の外部に、少なくとも5%の非 Bt ワタを refuge として栽培する。
② refuge のサイズは少なくとも 150 フィート（46m）幅（300 フィート幅が望ましい）とし、Bt ワタの栽培地の端から 1/2 マイル（0.8km）以内（隣接あるいは 1/4 マイル以内が望ましい）に設ける。
③このオプションを選択した場合、refuge にはいかなる殺虫剤も使用してはならない。
なお、このオプションは延長されない限り 2004 年の栽培年までの期限となっている。

オプション2：ほ場外における殺虫剤散布の refuge 設定

① Bt ワタの栽培地の外部に、少なくとも 20 %の非 Bt ワタを refuge として栽培する。
② refuge は Bt ワタの栽培地の端から 1 マイル（1.6km）以内（1/2 マイル以内が望ましい）に設ける。
③このオプションを選択した場合は、Bt 剤以外の殺虫剤、フェロモン剤等を併用してもよい。

オプション3：ほ場内における殺虫剤散布の refuge 設定

① Bt ワタの栽培地にブロック状に組み込む形で、少なくとも 5 %の非 Bt ワタを refuge として栽培する（ただし、ほ場の端に設置することは不可）。
② refuge のサイズは少なくとも 150 フィート（46m）幅（300 フィート幅が望ましい）とするが、標的害虫が Pink　bollworm だけの場合は Bt ワタ 6~10 列ごとに 1 列以上の refuge を栽培しても構わない。
③このオプションを選択した場合は、Bt 剤以外の殺虫剤、フェロモン剤等を併用してもよい。

（参考文献）

1）Biopesticides Registration Action Document: *Bacillus thuringiensis* (*Bt*) Plant-Incorporated Protectants (EPA October 15, 2001)
2）Carriere, Y. et al., J. Econ. Entomol. 94 (2): 315-325 (2001)

3　米国における **Bt** トウモロコシ（チョウ目害虫に対する **Cry 1Ab** および **Cry 1F**）の栽培基準

（1）コーンベルト地帯における飼料用 **Bt** トウモロコシの **refuge** 要件

少なくとも 20 ％の非 Bt トウモロコシを refuge として栽培する。refuge の栽培方法としては、

① Bt トウモロコシから 1/2 マイル（800m）以内（1/4 マイル以内が望ましい）の別のほ場に栽培

② Bt トウモロコシのほ場内にブロックを設定して栽培（ほ場の端に沿って、またはほ場の端に設定等）

③ほ場を帯状に横切るように最低 4 列幅（6 列幅が望ましい）で栽培

とする。どの場合も必要に応じて refuge に Bt 剤以外の殺虫剤を使用してもよい。

（2）ワタ栽培地域における飼料用 **Bt** トウモロコシの **refuge** 要件

少なくとも 50 ％の非 Bt トウモロコシを refuge として栽培する。標的害虫である Helicopvera zea はトウモロコシとワタの共通の害虫であり耐性害虫が発生しやすくなるため、広い refuge が求められる。refuge の栽培方法としては、

① Bt トウモロコシから 1/2 マイル（800m）以内（1/4 マイル以内が望ましい）の別のほ場に栽培

② Bt トウモロコシのほ場内にブロックを設定して栽培（ほ場の端に沿って、またはほ場の端に設定等）

③ほ場を帯状に横切るように最低 4 列幅（6 列幅が望ましい）で栽培

とする。どの場合も必要に応じて refuge に Bt 剤以外の殺虫剤を使用してもよい。

（3）**Bt** スイートコーン

Bt スイートコーンでは、特に refuge として非 Bt トウモロコシを栽培する必要はない。これは、スイートコーンは飼料用トウモロコシよりも収穫がかなり早く、収穫までには標的害虫であるチョウ目害虫の幼虫が成長し終わらないため、収穫後短期間のうちに植物体の残さを処分してしまえば、Bt 耐性を持った個体が生き残って越冬するのを防ぐことができるためである。Bt スイートコーンの植物体の残さの処分については、

①収穫後 30 日以内（14 日以内が望ましい）に行う

②方法はロータリー式刈り取り機で刈り取る、円盤鋤で耕す、あるいは鋤込む

こととする。

（参考文献）

1） Biopesticides Registration Action Document: *Bacillus thuringiensis* (*Bt*) Plant-Incorporated Protectants (EPA October 15, 2001)

4　栽培様式の模式図

National Corn Growers Association（全米トウモロコシ生産者協会）のホームページより引用

Bt Corn

Bt corn has proven to be an important technology to help corn growers control damaging insect pests and produce higher yields and better quality grain.

Insect Resistance Management (IRM)

To preserve the many benefits of Bt corn technology, the implementation of an IRM plan is essential. Experts agree, and government regulations require, that an effective Bt corn IRM plan includes the planting of a non-Bt refuge (a block of non-Bt corn) planted close to your Bt corn acres.

All Bt corn products designed to control European corn borer, southwestern corn borer and corn earworm require implementation of an IRM program according to the refuge size, distance guidelines and insecticide usage described in this fact sheet.

Growers who fail to follow these IRM requirements risk losing access to Bt corn technology.

Refuge Size Requirements

Corn-growing Areas (At Least 20% Refuge)

On each farm, plant at least 20 acres of non-Bt corn for every 80 acres of Bt corn (minimum of 20% non-Bt refuge, maximum of 80% Bt corn).

Corn/Cotton-growing Areas (At Least 50% Refuge)

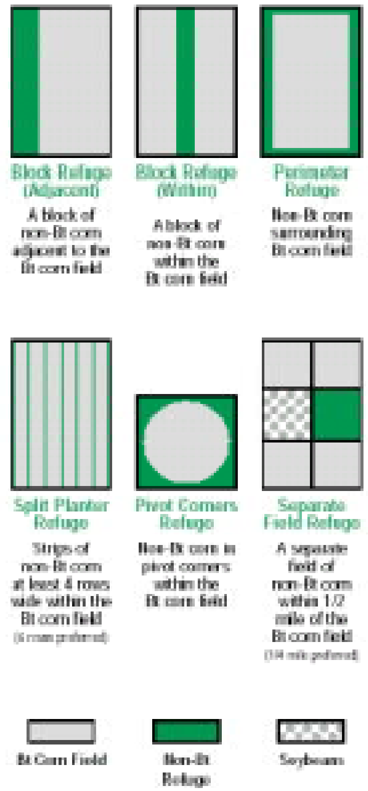
On each farm, plant at least 50 acres of non-Bt corn for every 50 acres of Bt corn (minimum of 50% non-Bt refuge, maximum of 50% Bt corn). See your seed company product use guide for the list of counties that fall under this requirement.

Refuge Distance Requirement

A non-Bt refuge must be planted within 1/2 mile of each Bt corn field, but preferably within 1/4 mile.

Refuge Planting Options

As illustrated below, the appropriate size non-Bt corn refuge may be planted a number of ways:



BIOPESTICIDES REGISTRATION ACTION DOCUMENT

***Bacillus thuringiensis* (*Bt*) Plant-Incorporated Protectants**

This version of the Biopesticides Registration Action Document for the *Bacillus thuringiensis* (*Bt*) Plant-Incorporated Protectants is dated October 15, 2001. This version corresponds to the version issued on September 29, 2001, with the following changes. The Agency has revised portions of Section I. Overview and Section II. Science Assessment relating to Cry1Ab and Cry1F proteins expressed in corn (*Bt* corn), in light of public comments received as of September 21, 2001. The Agency has also added two new sections entitled: "V. *Bt* Corn Confirmatory Data and Terms and Conditions of Amended Registration" and "VI. Regulatory Position on *Bt* Corn."

U.S. Environmental Protection Agency
Office of Pesticide Programs
Biopesticides and Pollution Prevention Division

Francis' Satyr butterfly and Kern Primrose Sphinx moth) are not going to be exposed to Cry1Ac protein because their habitats do not overlap with cotton fields.

Limited data do not indicate that Cry proteins have any measurable effect on microbial populations in the soil. Horizontal transfer from transgenic plants to soil bacteria has not been demonstrated. Purified microbially produced Cry1Ac protein produced a DT_{50} (Degradation Time) of 9.3-20.2 days. Ground, lyophilized Cry1A(c) cotton line 931tissue produced a DT_{50} of 41 days. Based upon estimates of 60,000 plants per acre, a total of 1.44 grams of Cry protein per acre would enter the soil when the cotton plants are incorporated after harvest.

3. Insect Resistance Management

Available data indicate that after six years of commercialization, no reported insect resistance has occurred to the *Bt* toxins expressed either in *Bt* potato, *Bt* corn, or *Bt* cotton products. The Agency believes that the existing IRM plan for *Bt* potato is adequate to mitigate Colorado potato beetle resistance. The existing IRM plan for *Bt* corn which had been strengthened for the 2000 growing season) was strengthened to further mitigate European corn borer, corn earworm, and southwestern corn borer. The existing IRM plan for *Bt* cotton (already strengthened for the 2001 growing season) was further strengthened to mitigate tobacco budworm, cotton bollworm, and pink bollworm resistance including requiring additional data to more closely examine the effectiveness of the 5% external, unsprayed refuge option.

The issue of insect resistance management has generated more data, meetings, and public comments than all of the other sections covered in this BRAD. Insect resistance management (IRM) is the set of practices aimed at reducing the potential for insect pests to become resistant to a pesticide. *Bt* IRM is of great importance because of the threat insect resistance poses to the future use of *Bt* plant-incorporated protectants and *Bt* technology as a whole. EPA considers protection of insect (pest) susceptibility of *Bt* to be in the "public good." EPA has determined that development of resistant insects would constitute an adverse environmental effect. In order to delay the development of insect resistance to *Bt* corn and cotton plant-incorporated protectants, EPA has mandated specific IRM requirements to strengthen the existing IRM programs as part of the terms and conditions of the registrations.

<u>a. *Bt* Corn</u>

The Agency has determined that the 20% non-*Bt* field corn refuge requirements for *Bt* corn grown in the Corn-Belt and the 50% non-*Bt* corn refuge requirements for *Bt* corn grown in cotton-growing areas are scientifically-sound, protective, feasible, sustainable, and practical to growers. Models have been developed by scientists in academia to predict the estimated time that insect resistance would develop to compare IRM strategies for *Bt* field corn. For example, if a high dose is achieved to control ECB (as it is for the currently registered *Bt* corn products), then these models predict that ECB will not evolve resistance for at least 99 years if a 20% refuge is implemented in the Corn Belt. Models are also used to predict the evolution of CEW resistance. These models indicate that 50% non-*Bt* field corn refuge in cotton-growing areas is sufficient to delay CEW resistance for at least the time frame of the registrations. A 20% non-*Bt* field corn refuge in the Corn Belt is sufficient to delay CEW resistance because CEW do not overwinter in the Corn Belt. EPA believes that the use of these models provides confidence that resistance will not evolve under the time frame of the registrations.

For *Bt* sweet corn, no specific refuge requirements are necessary because sweet corn is typically harvested much earlier than field corn, 18-21 days after silking, and before most lepidopteran larvae complete development. However, to mitigate the development of resistance, EPA has determined that crop residue destruction is necessary within 30 days. This practice will likely destroy any live larvae left in *Bt* sweet corn stalks and prevent overwintering of any resistant insects.

The IRM program for *Bt* field and sweet corn also require: 1) anyone purchasing *Bt* corn to sign a grower agreement which contractually binds the grower to comply with the IRM program and that there will be a mechanism by the year 2003 by which every grower affirms, annually, their contractual obligations to comply with the IRM program, 2) an IRM education program, 3) an IRM compliance monitoring program including a third party compliance survey and mechanisms to address non-compliance, 4) an insect resistance monitoring program for each target insect pest, 5) remedial action plans to be implemented if resistance does develop, and 6) annual reporting of the IRM (and other) activities. No other pesticide products than the *Bt* crop products have such extensive IRM requirements.

b. *Bt* Cotton

At this time, the Agency believes that available empirical data substantiate the success of the 5% external unsprayed, 20% external sprayed, and 5% embedded structured refuge options to delay resistance. However, EPA believes that it is imprudent to allow the 5% external, unsprayed refuge option for more than a limited period of time because current data indicates that this option has a significantly greater likelihood of insect resistance than either of the other refuge options. The 2000 SAP stated that the external, unsprayed option poses the highest risk to resistance evolution especially for cotton bollworm. Therefore, the external, unsprayed option expires after three growing seasons (September 30, 2004). During the next two years, the registrant is required to develop considerable new data on alternative host plants as possible effective refuges. In addition, the registrant is required to submit protocols by December 1, 2001, to begin field tests on alternative hosts and chemical insecticide sprays on *Bt* cotton, and to provide annual reports each January 31st. If any of these terms and conditions are not met, the external, unsprayed refuge option will be eliminated. If, based upon these, and any other pertinent data, the registrant requests an amendment to the registration extending the expiration date of the external, unsprayed option, EPA will conduct a comprehensive assessment of whether all relevant data support such regulatory action, as part of a larger requirement that would also likely involve alternative host plants.

In addition, the Agency is mandating additional improvements to the current IRM programs that will require: 1) anyone purchasing *Bt* cotton to sign a grower agreement which contractually binds the grower to comply with the IRM program and that there will be a mechanism by the year 2003 by which every grower affirms their contractual obligations to comply with the IRM program, 2) an ongoing IRM education program, 3) an ongoing IRM compliance monitoring program including a third party compliance survey and mechanisms to address non-compliance, 4) and ongoing insect resistance monitoring program for each target insect pest, 5) remedial action plans to be implemented if resistance does develop, and 6) annual reporting of the IRM (and other) activities. No other pesticide products than the *Bt* crop products have such extensive IRM requirements.

4. Benefits

EPA believes that significant benefits accrue to growers, the public, and the environment from the availability and use of certain *Bt* plant-incorporated protectants. This section outlines how those benefits are defined and evaluated. Specific information on grower cost savings, increased yields, reduced conventional pesticide use, benefits to wildlife, etc. is presented by product. Direct benefits to growers for all *Bt* products is estimated to be less than $350 million in 2000. Major environmental benefits occur through less insecticide use and improved product quality.

a. *Bt* Corn

In addition to assessing the risks from the use of Cry1Ab and Cry1F expressed in corn, EPA has evaluated the benefits from the use of these products. Direct grower benefits include improved yield and profitability, improved crop management effectiveness, reduction in farming risk, and improved opportunity to grow field corn in case of severe pest infestation. Total annual monetary grower benefits from the use of Bt field corn are less than $219 million annually. The magnitude of benefits for any year is largely a function of the level of lepidopteran insect pressure in that year. That is, other things being equal, the higher the insect pressure, the higher the benefits. The major environmental benefit is potential reduction in mycotoxins. EPA believes that use of Bt sweet corn would result in significant reductions in the use of chemical pesticides. However, the current use of Bt sweet corn is very low.

b. *Bt* Cotton

In addition to assessing the risks from the use of Cry1Ac expressed in cotton, EPA has evaluated the benefits from the use of this product. Direct grower benefits include reduced pesticide use, improved crop management effectiveness, reduced production costs, improved yield and profitability, reduction in farming risk, and improved opportunity to grow cotton in areas of severe pest infestation. Total monetary grower benefits from the use of Bt cotton are between $60 million

a. No planting of *Bt*-cotton south of Route 60 (near Tampa) in Florida,
b. Commercial culture of *Bt*-cotton is prohibited in the state of Hawaii,
c. Test plots or breeding nurseries established in Hawaii must be surrounded by 24 border rows of a suitable pollinator trap crop regardless of the plot size and must not be planted within 3 miles of *Gossypium tomentosum*,
d. Commercial culture, experimental plots and breeding nurseries of Bt.-cotton are prohibited in the U.S. Virgin Islands, and
e. Commercial culture of Bollgard™ cotton is prohibited in Puerto Rico. Test plots or breeding nurseries established on the island of Puerto Rico must be surrounded by 24 border rows of a suitable pollinator trap crop regardless of the plot size and must not be planted within 3 miles of feral cotton plants.

Upon approval by EPA, test plots and/or breeding nurseries in Hawaii, the U.S. Virgin Islands, and Puerto Rico may be established without restrictions if alternative measures, such as insecticide applications, are shown to effectively mitigate gene flow.

c. Insect Resistance Management (IRM) Program

i. *Bt* Corn

The Agency has determined that the unrestricted use of Cry1Ab and/or Cry1F in corn is likely to lead to the emergence of resistance in one or more of the target insect pests unless measures are used to delay or halt the development of resistant insects. Because some corn pests also attack other crops, not only would the emergence of resistance affect the benefits of *Bt* corn, such insect resistance could also affect the efficacy of *Bt* cotton products and microbial formulations of *Bt*. The loss of *Bt* as an effective pest management tool – in field corn, sweet corn, or other crops – could potentially have serious adverse consequences for the environment to the extent that growers might shift to the use of more toxic pesticides and a valuable tool for organic farmers might be lost. The emergence of resistance in corn pests could also have significant economic consequences for corn growers. Therefore, EPA continues to require the registrants to implement an Insect Resistance Management (IRM) program to mitigate the possibility that pest resistance will occur.

The required IRM program for *Bt* corn has the following elements:

1] Requirements relating to creation of a non-*Bt* corn refuge in conjunction with the planting of any acreage of *Bt* field corn;

2] Requirements for the registrants to prepare and require *Bt* corn users to sign “grower agreements” which impose binding contractual obligations on the grower to comply with the refuge requirements;

3] Requirements for the registrants to develop, implement, and report to EPA on programs to educate growers about IRM requirements;

4] Requirements for the registrants to develop, implement, and report to EPA on programs to evaluate and promote growers' compliance with IRM requirements;

5] Requirements for the registrants to develop, implement, and report to EPA on programs to evaluate whether there are statistically significant and biologically relevant changes in target insect susceptibility to Cry1Ab protein and/or Cry1F in the target insects;

6] Requirements for the registrants to develop, and if triggered, to implement a "remedial action plan" which would contain measures the registrants would take in the event that any insect resistance was detected as well as to report on activity under the plan to EPA;

7] Submit annual reports on sales, IRM grower agreements results, compliance, and educational program on or before January 31st each year.

a. Refuge Requirements

1) Field Corn

a) Corn-Belt Refuge Requirements

For Cry1Ab and Cry1F *Bt* field corn grown outside cotton-growing areas (e.g., the Corn Belt), grower agreements (also known as stewardship agreements) will specify that growers must adhere to the refuge requirements as described in the grower guide/product use guide and/or in supplements to the grower guide/product use guide.

- Specifically, growers must plant a structured refuge of at least 20% non-*Bt* corn that may be treated with insecticides as needed to control lepidopteran stalk-boring and other pests.

- Refuge planting options include: separate fields, blocks within fields (e.g., along the edges or headlands), and strips across the field.

- External refuges must be planted within ½ mile (1/4 mile or closer preferred).

- When planting the refuge in strips across the field, refuges must be at least 4 rows wide, preferably 6 rows wide.

- Insecticide treatments for control of ECB, CEW and Southwestern corn borer (SWCB) [Cry1Ab or Cry1F corn hybrids] and/or fall armyworm (FAW) and black cutworm (BCW) [Cry1F corn hybrids only] may be applied only if economic thresholds are reached for one or more of these target pests. Economic thresholds will be determined using methods recommended by local or regional professionals (e.g., Extension Service agents, crop consultants). Instructions to growers will specify that microbial *Bt* insecticides must not be applied to non-*Bt* corn refuges.

b) Cotton-Growing Area Refuge Requirements for *Bt* Corn

For Cry 1Ab and Cry1F *Bt* field corn grown in cotton-growing areas, grower agreements (also known as stewardship agreements) will specify that growers must adhere to the refuge requirements as described in the grower guide/product use guide and/or in supplements to the grower guide/product use guide.

- Specifically, growers in these areas must plant a structured refuge of at least 50% non-*Bt* corn that may be treated with insecticides as needed to control lepidopteran stalk-boring and other pests.

- Refuge planting options include: separate fields, blocks within fields (e.g., along the edges or headlands), and strips across the field.

- External refuges must be planted within ½ mile (1/4 mile or closer preferred).

- When planting the refuge in strips across the field, refuges must be at least 4 rows wide, preferably 6 rows wide.

- Insecticide treatments for control of ECB, CEW and Southwestern corn borer (SWCB) [Cry1Ab or Cry1F corn hybrids] and/or fall armyworm (FAW) and black cutworm (BCW) [Cry1F corn hybrids only] may be applied only if economic thresholds are reached for one or more of these target pests. Economic thresholds will be determined using methods recommended by local or regional professionals (e.g., Extension Service agents, crop consultants). Instructions to growers will specify that microbial *Bt* insecticides must not be applied to non-*Bt* corn refuges.

- Cotton-growing areas[1] include the following states: Alabama, Arkansas, Georgia, Florida, Louisiana, North Carolina, Mississippi, South Carolina, Oklahoma (only the counties of Beckham, Caddo, Comanche, Custer, Greer, Harmon, Jackson, Kay, Kiowa, Tillman, Washita), Tennessee (only the counties of Carroll, Chester, Crockett, Dyer, Fayette, Franklin, Gibson, Hardeman, Hardin, Haywood, Lake, Lauderdale, Lincoln, Madison, Obion, Rutherford, Shelby, and Tipton), Texas (except the counties of Carson, Dallam, Hansford, Hartley, Hutchinson, Lipscomb, Moore, Ochiltree, Roberts, and Sherman), Virginia (only the

[1]Counties selected based on approximately 1000 A *Bt* cotton/5000 A total cotton using 1999-2001 cotton acreage reports from Monsanto and USDA/NASS.

counties of Dinwiddie, Franklin City, Greensville, Isle of Wight, Northampton, Southampton, Suffolk City, Surrey, Sussex) and Missouri (only the counties of Dunkin, New Madrid, Pemiscot, Scott, Stoddard). The correct list of counties must be in the 2003 grower guide and may be provided as a supplement for the 2002 growing season.

b. Sweet Corn Post-Harvest Requirements

Sweet corn is harvested long before field corn. Therefore, if the sweet corn stalks remaining in the field and any insects remaining in the stalks are destroyed shortly after harvest, a refuge is not needed as a part of the IRM program for sweet corn. Growers must adhere to the following types of crop destruction requirements as described in the grower guide/product use guide and/or in supplements to the grower guide/product use guide.

- Crop destruction must occur no later than 30 days following harvest, but preferably within 14 days.
- The allowed crops destruction methods are: rotary, mowing, discing, or plow-down. Crop destruction methods should destroy any surviving resistant insects.

ii. *Bt* Cotton

The Agency has determined that the unrestricted use of Cry1Ac as expressed in cotton is likely to lead to the emergence of resistance in one or more of the target insect pests unless measures are used to delay or halt the development of resistant insects. EPA is requiring the registrant to implement an Insect Resistance Management (IRM) program to mitigate the possibility that pest resistance will occur. The required IRM program for *Bt* cotton has the following elements:

1] Requirements relating to creation of a non-*Bt* cotton refuge in conjunction with the planting of any acreage of *Bt* cotton;

2] Requirements for the registrant to prepare and require *B*t cotton users to sign "grower agreements" which impose binding contractual obligations on the grower to comply with the refuge requirements;

3] Requirements for the registrant to develop, implement, and report to EPA on programs to educate growers about IRM requirements;

4] Requirements for the registrant to develop, implement, and report to EPA on programs to evaluate and promote growers' compliance with IRM requirements;

5] Requirements for the registrant to develop, implement, and report to EPA on programs to evaluate whether there are statistically significant and biologically relevant changes in susceptibility to Cry1Ac protein in the target insects;

6] Requirements for the registrant to develop, and if triggered, to implement a "remedial action plan" which would contain measures the registrant would take in the event that any insect resistance was detected as well as to report on activity under the plan to EPA;

7] Submit annual reports on or before January 31st each year.

All growers of *Bt* cotton must employ one of the following structured refuge options:

External, Unsprayed Refuge

Ensure that at least 5 acres of non-*Bt* cotton (refuge cotton) is planted for every 95 acres of *Bt* cotton. The size of the refuge must be at least 150 feet wide, but preferably 300 feet wide. This refuge may not be treated with sterile insects, pheromones, or any insecticide (except listed below) labeled for the control of tobacco budworm, cotton bollworm, or pink bollworm. The refuge may be treated with acephate or methyl parathion at rates which will not control tobacco budworm or the cotton bollworm (equal to or less than 0.5 lbs active ingredient per acre). The variety of cotton planted in the refuge must be comparable to *Bt* cotton, especially in the maturity date, and the refuge must be managed (e.g., planting time, use of fertilizer, weed control, irrigation, termination, and management of other pests) similarly to *Bt* cotton. Ensure that a non-*Bt* cotton refuge is maintained within at least ½ linear mile (preferably adjacent to or within 1/4 mile or closer) from the *Bt* cotton fields. This option expires after the 2004 growing season unless extended by amendment as described below. EPA intends to review the data specified in the data requirements concerning alternate hosts and chemical insecticide sprays applied to *Bt* cotton, and decide in 2004 whether the new data support continuation of an external, unsprayed refuge as part of a larger requirement that would also likely involve alternative host plants. If these data support the continued availability of the external, unsprayed refuge option, EPA may approve an amendment to this registration to maintain the availability of this option.

External Sprayed Refuge

Ensure that at least 20 acres of non-*Bt* cotton are planted as a refuge for every 80 acres of *Bt* cotton (total of 100A) . The variety of cotton planted in the refuge must be comparable to *Bt* cotton, especially in the maturity date, and the refuge must be managed (e.g., planting time, use of fertilizer, weed control, irrigation, termination, and management of other pests) similarly to *Bt* cotton. The non-*Bt* cotton may be treated with sterile insects, insecticides (excluding foliar *Btk* products), or pheromones labeled for control of the tobacco budworm, cotton bollworm, or pink bollworm. Ensure that a non-*Bt* refuge is maintained within at least 1 linear mile (preferably within ½ mile or closer) from the *Bt* cotton fields.

Embedded Refuge

Plant at least 5 acres of non-*Bt* cotton (refuge cotton) for every 95 acres of *Bt* cotton. The refuge cotton must be embedded as a contiguous block within the Bt cotton field, but not at one edge of the field (i.e., refuge block(s) surrounded by *Bt* cotton). For very large fields, multiple blocks across the field may be used. For small or irregularly shaped fields, neighboring fields farmed by the same grower can be grouped into blocks to represent a larger field unit, provided the block exists within one mile squared of the Bt cotton and the block is at least 150 feet wide, but preferably 300 feet wide. Within the larger field unit, one of the smaller fields planted to non-Bt cotton may be utilized as the embedded refuge. The variety of cotton planted in the refuge must be comparable to *Bt* cotton, especially in the maturity date, and the refuge must be managed (e.g., planting time, use of fertilizer, weed control, irrigation, and management of other pests) similarly to *Bt* cotton. This refuge may be treated with sterile insects, any insecticide (excluding foliar *Btk* products), or pheromones labeled for the control of tobacco budworm, cotton bollworm, or pink bollworm whenever the entire field is treated. The refuge may not be treated independently of the surrounding *Bt* cotton field in which it is embedded (or fields within a field unit).

Embedded Refuge for Pink Bollworm Only

Plant the refuge cotton as at least one single non-*Bt* cotton row for every six to ten rows of *Bt* cotton. The refuge may be treated with sterile insects, any insecticide (excluding foliar *Btk* products), or pheromones labeled for the control of pink bollworm whenever the entire field is treated. The in-field refuge rows may not be treated independently of the surrounding *Bt* cotton field in which it is embedded. The refuge must be managed (fertilizer, weed control, etc.) identically to the *Bt* cotton. There is no field unit option.

Optional Community Refuge Pilot

This option allows multiple growers to manage refuge for external, unsprayed and external, sprayed refuge options or both. This option is not allowed for the embedded/in-field options. A community refuge program will be allowed as a continuing pilot for the 2002 growing season. The community refuge for insect resistance management must meet the requirements of either the 5% external unsprayed refuge and/or the 20% sprayed option, or an appropriate combination of the two options. The registrant must implement the 2002 community refuge pilot program as described in the Bollgard® Cotton 2002 Refuge Guide.

7. Regulatory Position on *Bt* Corn

EPA's finding that Cry1Ab or Cry1F protein expressed in corn will not significantly increase the risk of unreasonable adverse effects on the environment is based on the analysis contained in the succeeding sections of this BRAD and the specific terms and conditions that are imposed upon this registration, as set forth in Section V. In general terms, EPA concludes that use of Cry1Ab or Cry1F as expressed in corn is effective at controlling significant lepidopteran pests of corn including European corn borer, corn earworm, and southwestern corn borer. Therefore, these products have

a. Elements of IRM Plans

To address the very real concern of insect resistance to *Bt* proteins, EPA has imposed IRM requirements on registered *Bt* plant-pesticides. Sound IRM will prolong the life of *Bt* pesticides and adherence to the plans is to the advantage of growers, producers, researchers, and the American public. EPA considers the development of *Bt*-resistant insects to constitute an adverse environmental effect. EPA's strategy to address insect resistance to *Bt* is two-fold: 1) mitigate any significant potential for pest resistance development in the field by instituting IRM plans, and 2) better understand the mechanisms behind pest resistance.

Scientific experts believe that a high dose and the planting of a refuge (a portion of the total acreage using non-*Bt* seed) will delay the development of insect resistance to *Bt* crops by maintaining insect susceptibility. In addition to a high dose and structured refuge, IRM plans include additional field research on pest biology, refuge size and deployment, resistance monitoring for the development of resistance (and increased insect tolerance of the protein), grower education, a remedial action plan in case resistance is identified, annual reporting and communication. IRM plans will change as more scientific data become available.

Beginning with the first *Bt* plant-pesticide registration, the Agency has taken steps to manage insect resistance to *Bt* with IRM plans being an important part of the regulatory decision. The Agency identified (later confirmed by the 1995 SAP) seven elements that should be addressed in a *Bt* plant-incorporated protectant resistance management plan: 1) knowledge of pest biology and ecology; 2) appropriate dose expression strategy; 3) appropriate refuge; 4) resistance monitoring and a remedial action plan should resistance occur; 5) employment of integrated pest management (IPM); 6) communication and education strategies on use of the product; and 7) development of alternative modes of action. IRM plans also include grower education and measurement of the level of compliance. Because IRM plans change as more scientific data become available, EPA has also imposed research data requirements as part of the terms and conditions of registration. EPA has also made changes to IRM requirements as the science has evolved.

b. High Dose/Structured Refuge Strategy

The 1998 Science Advisory Panel Subpanel agreed with EPA that an appropriate resistance management strategy is necessary to mitigate the development of insect resistance to *Bt* proteins expressed in transgenic crop plants. The 1998 Subpanel recognized that resistance management programs should be based on the use of both a high dose of *Bt* and structured refuges designed to provide sufficient numbers of susceptible adult insects. The high dose/refuge strategy assumes that resistance to *Bt* is recessive and is conferred by a single locus with two alleles resulting in three genotypes: susceptible homozygotes (SS), heterozygotes (RS), and resistant homozygotes (RR). It also assumes that there will be a low initial resistance allele frequency and that there will be extensive random mating between resistant and susceptible adults. Under ideal circumstances, only rare RR individuals will survive a high dose produced by the *Bt* crop. Both SS and RS individuals will be susceptible to the *Bt* toxin. A structured refuge is a non-*Bt* portion of a grower's field or set of fields that provides for the production of susceptible (SS) insects that may randomly mate with rare resistant (RR) insects surviving the *Bt* crop to produce susceptible RS heterozygotes that will be killed by the *Bt* crop. This will remove resistant (R) alleles from the insect populations and delay the evolution of resistance. The 1998 and 2000 SAP Subpanels noted that insect resistance management strategies should also be sustainable and to the extent possible, strongly consider grower acceptance and logistical feasibility.

Although the high dose/refuge strategy is the preferred strategy for IRM, effective IRM is still possible even if the transformed plant does not express the *Bt* protein at a high dose for all economically-important target pests (e.g., by increasing refuge size). The lack of a high dose could allow partially resistant (i.e. heterozygous insects with one resistance allele) to survive, thus increasing the frequency of resistance genes in an insect population. For this reason, numerous IRM researchers and expert groups have concurred that non-high dose *Bt* expression presents a substantial resistance risk relative to high dose expression (Roush 1994, Gould 1998, Onstad & Gould 1998, SAP 1998, ILSI 1998, UCS 1998, SAP 2001). The 1998 SAP Subpanel also noted that insect resistance management strategies should be sustainable and to the extent possible, strongly consider grower acceptance and logistical feasibility.

The 1998 SAP Subpanel defined (and the 2000 SAP Subpanel confirmed) a high dose as "25 times the protein concentration necessary to kill susceptible larvae." The logic for this approach is spelled out in the 1998 SAP report as well as in the scientific literature on insect resistance management for *Bt* crops. In essence, *Bt* cultivars must produce a high enough toxin concentration to kill nearly all of the insects that are heterozygous for resistance. The Agency has adopted the 25X definition of high dose proposed by the 1998 SAP Subpanel.

The 1998 SAP Subpanel noted that a *Bt* plant-incorporated protectant could be considered to provide a high dose if verified by at least two of the following five approaches: 1) Serial dilution bioassay with artificial diet containing lyophilized tissues of *Bt* plants using tissues from non-*Bt* plants as controls; 2) Bioassays using plant lines with expression levels approximately 25-fold lower than the commercial cultivar determined by quantitative ELISA or some more reliable technique; 3) Survey large numbers of commercial plants in the field to make sure that the cultivar is at the $LD_{99.9}$ or higher to assure that 95% of heterozygotes would be killed (see Andow & Hutchison 1998); 4) Similar to #3 above, but would use controlled infestation with a laboratory strain of the pest that had an LD_{50} value similar to field strains; and 5) Determine if a later larval instar of the targeted pest could be found with an LD_{50} that was about 25-fold higher than that of the neonate larvae. If so, the later stage could be tested on the *Bt* crop plants to determine if 95% or more of the later stage larvae were killed. The 2000 SAP concluded that the current *Bt* potato and *Bt* corn have *Bt* titers that will significantly exceed the 25X criteria for control of Colorado potato beetle and European corn borer, respectively. In terms of *Bt* cotton, the 2000 SAP concluded that "all cotton cultivars in the U.S. probably produced a high dose" for

TBW and PBW, while "none of the cultivars produce a high dose" for CBW.

As an alternate definition for high dose, Caprio et al. (2000) recommend that a higher, 50-fold value be adopted (rather than 25-fold) because current empirical data suggest that a 25-fold dose may not be consistently high enough to cause high mortality among heterozygotes with known *Bt* resistance alleles. The 2000 SAP Subpanel did not recommend changing the existing 25-fold definition, but noted that the "25X" definition is imprecise, provisional, and may require modification as more knowledge becomes available about the inheritance of resistance. The Subpanel concluded that current *Bt* corn and *Bt* cotton varieties have less than a 25-fold dose for CBW.

The size, placement, and management of the refuge is critical to the success of the high dose/structured refuge strategy to mitigate insect resistance to the *Bt* proteins produced in corn, cotton, and potatoes. The 1998 Subpanel defined structured refuges to "include all suitable non-*Bt* host plants for a targeted pest that are planted and managed by people. These refuges could be planted to offer refuges at the same time when the *Bt* crops are available to the pests or at times when the *Bt* crops are not available." The 1998 Subpanel suggested that a production of 500 susceptible adults in the refuge for every adult in the transgenic crop area (assuming a resistance allele frequency of 5×10^{-2}) would be a suitable goal. The placement and size of the structured refuge employed should be based on the current understanding of the pest biology data and the technology. The 1998 SAP Subpanel also recognized that refuges should be based on regional pest control issues. The 2000 SAP Subpanel echoed the 1998 SAP's recommendations that the refuge should produce 500:1 susceptible to resistant insects and that regional IRM working groups would be helpful in developing policies.

c. Predictive Models

EPA has used predictive models to compare IRM strategies for *Bt* crops. Because models cannot be validated without actual field resistance, models have limitations and the information gained from the use of models is only a part of the weight of evidence used by EPA in assessing the risks of resistance development. It was the consensus of the 2000 SAP Subpanel that models were an important tool in determining appropriate *Bt* crop IRM strategies. They agreed that models were "the only scientifically rigorous way to integrate all of the biological information available, and that without these models, the Agency would have little scientific basis for choosing among alternative resistance management options." They also recommended that models must have an agreed upon time frame for resistance protection. For example, conventional growers may desire a maximum planning horizon of five years, while organic growers may desire an indefinite planning horizon. The Subpanel recommended that model design should be peer reviewed and parameters validated. Models should also include such factors as level of *Bt* crop adoption, level of compliance, economics, fitness costs of resistance, alternate hosts, spatial components, stochasticity, and pest population dynamics.