

**Review of the GM Inspection and Enforcement Regime
Covering the Deliberate Release of Genetically Modified
Plants under Part B of Directive 2001/18/EC**

The Advisory Committee on Releases to the Environment

April 2003

1. Introduction, background and scope

- 1.1 The release of genetically modified organisms¹ (GMOs) in Europe is controlled by Directive 2001/18/EC². The Directive provides a European Community-wide regime so that no GMOs may be released or marketed in the Community without consent from the regulatory authorities. Applicants for consent to release must supply a dossier of prescribed information³ about the GMO, and this should include a detailed risk assessment of its possible impact on human health and the environment.
- 1.2 In the UK, the Advisory Committee on Releases to the Environment (ACRE) reviews all applications to release and market GMOs and advises Ministers⁴ on the risks to human health and the environment that would result from the releases. The great majority of ACRE's caseload currently involves the assessment of risks associated with release of GM plants. The releases can be for the purposes of research and development or for marketing.
- 1.3 During the summer of 2002 there was a well publicised incident where it was revealed that, at certain release sites in the programme of Farm Scale Evaluations (FSEs) of GM crops, GM material in addition to that for which consent to release was held had been present. At this point ACRE considered the risks posed by the specific additional GM material, and concluded 'that the presence of the additional transformation events did not pose any additional risks to human health or the environment'⁵.
- 1.4 As a result of this incident ACRE was asked to review the current regime for inspection and enforcement of the GM regulations from the point of view of risk assessment. Specifically the Committee was asked to consider what, if any, additional measures were required to ensure that any risks associated with the release of GMOs are adequately managed.
- 1.5 In order to carry out this review ACRE established a working group. The terms of reference and membership of the group are listed in Annex 1. In addition to considering the implications of the FSE seed impurity incident, ACRE also asked the GM Inspectorates for England and Wales, and Scotland⁶ to highlight any issues concerning the inspection and enforcement regime which they would like the review to consider in the light of the Inspectorates experience in carrying out the regime over recent years.
- 1.6 As outlined above, the central feature of the regulatory regime for the release of GMOs is a thorough case-by-case risk assessment which ensures that only those GMOs that are considered to pose no additional risk to human health and the environment are released. This process also ensures that any risk management measures (e.g. separation distances, control of volunteers) are appropriate. Following this process, the consent holder has three main responsibilities, currently defined as:

¹ The term "genetically modified organism" used in this document refers to the definition used in section 106 of the Environmental Protection Act 1990.

² In the UK, Directive 2001/18/EC is implemented by the Genetically Modified Organisms (Deliberate Release) 2002, and Part VI of the Environmental Protection Act 1990.

³ Annex III of Directive 2001/18/EC

⁴ UK Government and Devolved Administrations of Scotland, Wales and Northern Ireland

⁵ The full text of ACRE's advice can be found at <http://www.defra.gov.uk/environment/acre/advice/advice17.htm>

⁶ The GM Inspectorate for England and Wales is located at the Central Science Laboratory (York), while the GM Inspectorate for Scotland is located at the Scottish Agricultural Science Agency (Edinburgh).

- i. To ensure that only the GMO for which risk assessment has been carried out is released.
 - ii. To ensure that any required risk management measures are implemented appropriately.
 - iii. To monitor for unexpected consequences of the release, and act appropriately to manage any unanticipated risks.
- 1.7 In order to ensure that consent holders carry out their responsibilities it is necessary to monitor their compliance – this is the purpose of the GM inspection and enforcement regime.
- 1.8 The considerations of ACRE reported here are restricted to specific types of release. First, we have limited our considerations to releases for the purposes other than marketing (Part B releases) – the group will consider releases for the purposes of marketing (Part C releases) at a later date. Secondly, only releases of crop plants are considered, not GM animals or micro-organisms. Where appropriate, it is necessary to consider individual crop species separately, since the risks, and consequently the risk management measures, can vary considerably.
- 1.9 We have employed the following guiding principles in our analysis of the issues surrounding inspection and enforcement of Part B releases:
 - Only releases of GM crops that, given the proposed risk management measures, are deemed to be of no additional risk compared to their conventional counterpart will be approved
 - The aim of inspection and enforcement is to protect human health and the environment by ensuring that consent holders fulfil their responsibilities listed in paragraph 1.6
 - The inspection and enforcement regime should be proportionate to the risks concerned
 - Consent holders should only be required to carry out activities that are necessary to protect human health and the environment
- 1.10 In this report, three issues will be considered: the purpose and scale of Part B releases, the steps required to ensure that only the GMO for which risk assessment has been carried out is released, and, the steps required to check compliance of consent holders with risk management and monitoring requirements.

2. Purpose and scale of Part B releases

- 2.1 Directive 2001/18 defines Part B releases as ‘Deliberate release of GMOs for any other purpose than for placing on the market’. Primarily this equates to releases of genetically modified crops for the purposes of research, which covers a number of possible purposes. Some Part B releases are of crops that are in the final stages of development prior to commercialisation. In this case the purpose of the trial is usually to monitor agronomic performance under a range of conditions, and would include trials carried out under the National Seed listing procedures⁷. For this type of Part B release it is usual to only release a material containing a single distinct transformation event, although there may be multiple varieties carrying the same transformation event (i.e. the same GM material in different genetic backgrounds).
- 2.2 In contrast, other releases under Part B will be of GM plants at much earlier stages of research prior to commercialisation, which can also include releases for purely academic ‘blue-skies’ research. In this case it is often necessary for the purposes of the work to release multiple distinct transgenic lines. These lines will contain the same GM material, but inserted in different parts of the genome.
- 2.3 A common feature of almost all Part B releases is that they are carried out on a small scale. They are generally less than 1 hectare (2.5 acres), and are often much less than this. Trial releases of as little as 0.1 hectares (0.25 acres) are common. This small scale of release has important implications for risk management. For example, it is possible to control volunteers on plots of this size very easily by hand, and, as the plots are often located at research stations, they are regularly inspected by the researchers carrying out the work. A further key point concerning these releases is that only small amounts of seed (or other propagules) are required, so that these can be produced in carefully controlled conditions, usually of full containment.
- 2.4 An exception to the generally small-scale releases carried out under Part B has been the Farm Scale Evaluations (FSEs). As the name implies, these experiments required cultivation of GM crops on a comparatively large area, often several hectares, raising issues for risk management. As well as the large size of individual FSE plots, there were also a large number of sites across the UK. This was important for the objectives of the FSE experiment, so that representative and statistically robust data could be obtained. A consequence of this was that significant quantities of seed were required for these sites that could only be produced using commercial-scale seed production facilities.
- 2.5 Clearly, the FSEs provide additional challenges concerning inspection and enforcement compared to more typical Part B releases. However, it is important to note that the FSEs are virtually completed – the final crop of winter oilseed rape will be harvested in the early summer of 2003. As a result we have concentrated our considerations on the requirements for inspection and enforcement for typical, small-scale research releases.
- 2.6 It is unlikely that any programme of work involving GM crop releases on the scale of the FSEs under a Part B consent will be contemplated in the future. However, if such an application for a Part B consent were received, it would be important for ACRE to consider the risk management and inspection and enforcement requirements for such a release on a case-by-case basis. It may be that such a release would only be recommended if specific additional conditions were attached to the consent.

⁷ See <http://www.defra.gov.uk/planth/pvs/default.htm> for details.

3. Confirming the nature of the GMO released

- 3.1 As outlined in paragraph 1.6, one of the responsibilities of consent holders is to ensure that only the GMO for which risk assessment has been carried out is released. This is important because each risk assessment is carried out on a case-by-case basis, so if a different GM plant is released, then the risks may be different. It is important to note that, for Part B releases, the central issue for risk assessment is the nature of the inserted genes, rather than the location of this insertion into the genome. This is because the release is small scale and there is no intention for the GM crop to enter the food or feed chain.
- 3.2 The current approach to confirming that consent holders are satisfying their responsibility in this regard is to audit their trial management procedures, which, *inter alia*, involves a consideration of the procedures for the production of seeds and other propagules. Currently, consent holders are required to demonstrate that they have procedures in place to ensure that the correct GMO is released, and this may include the examination of test data produced by the consent holder. There is no requirement on the consent holder to provide confirmatory data before the release, and there is no random testing by the GM Inspectorates before or after the start of the release. It is also not routine practice for the inspectorate to take samples.
- 3.3 Clearly the recent incident where additional GM material was identified at FSE sites (see paragraph 1.3) suggests that procedures may need to be revised. However, it is important to note that the oilseed rape seed used for this part of the FSEs was produced in large scale commercial seed production facilities. Under normal circumstances, the seed or other propagules required for Part B releases would be prepared under contained conditions where steps are taken to ensure genetic isolation⁸, and so the chance of the presence of impurities is very low.
- 3.4 The likelihood of seed or other propagules containing additional GM material is also dependent on the crop species under consideration. For example, plants that are vegetatively propagated (e.g. potato tubers) will not acquire additional GM material through cross pollination, so the chances of unexpected GM presence even outside of contained conditions is very much lower than for crops that are propagated through seed.
- 3.5 The Committee would, therefore, **recommend** different inspection procedures depending on the conditions under which seed or other propagules are produced, and the nature of the propagule (seed vs. vegetative). A detailed description of the conditions under which seeds are to be produced should be included in applications for consent to release.

Seed production under conditions of genetic isolation or vegetative propagules

- 3.6 For seed produced under contained conditions the existing system of management audits is generally sufficient. This is also the case for releases of plants that are propagated vegetatively.

⁸ A minimum standard required for genetic isolation would be seed/propagule production under contained conditions. For species where cross pollination is possible, then additional measures will also be required, such as physical isolation from other sexually compatible GMOs, or emasculation and hand pollination.

- 3.7 If seeds or other propagules are produced under conditions of genetic isolation or the propagules are vegetative then the Committee **recommends** that the present system of management audits should be maintained.

Seed production without measures to ensure genetic isolation

- 3.8 Where seed have been produced without measures to ensure genetic isolation it is necessary to take additional steps to confirm that the consent holders have taken sufficient care to ensure that seed purity has been maintained. In this case the Inspectorate should be provided with test data by the consent holder confirming the absence of additional GM material from the seed batches to be used in the release, together with a seed sample. The Inspectorate should carry out independent tests for the presence of GM material in this sample for at least a proportion of consents in order to independently validate the test data provided by the consent holder. It is important to emphasise that no inspection regime can provide an absolute guarantee that there is no additional GM material – the aim should be to provide reassurance that the probability of additional GM presence is low.
- 3.9 It is envisaged that the majority of tests carried out in order to confirm presence and absence of specific DNA sequences in GM material will be PCR-based. However, alternative approaches (e.g. herbicide tolerance tests) may also be appropriate under some circumstances. The scale of testing should be proportionate to the risks concerned, and should focus on DNA sequences (coding regions and regulatory sequences) that are present in GM crops that are grown commercially in the region where seed production has been carried out. For each GM plant to be released a small number of sequences found in GM plants should be selected using the following criteria:
- Sequences are expected to be absent from the GM plant to be released
 - Sequences are found in GM crops that are cultivated commercially in the region where seed has been produced
- 3.10 Given the current range of GM crops that are grown commercially, testing for usually no more than 3 sequences that should be absent from the GM plant to be released should give a reasonable level of assurance concerning seed purity⁹. Clearly, the sequences chosen must be determined on a case-by-case basis, and the number of tests carried out should be reviewed as the use of GM crops expands worldwide. It is important to note that all tests have limits of detection, so can only provide evidence that the presence of a particular sequence is below that limit, rather than absent (see Annex 2 for details).
- 3.11 The tests carried out by the consent holder should be of a sufficient quality to establish that sequences for which tests are carried out are not present above a stated threshold. Confirming that a sequence is not present requires careful experimentation with particular attention to designing suitable control experiments¹⁰. It is recommended that absence of a sequence is confirmed by using two independent primer pairs for each

⁹ Testing for the presence of 3 additional GM sequences should, in most cases, be adequate. However, it may be necessary to increase the number of sequences tested for on a case-by-case basis, and ACRE will consider this as part of their assessment of Part B applications.

¹⁰ The issue of providing evidence that specific DNA sequences are absent is considered in ACRE's recent report on *Guidance on best practice for the presentation and use of molecular data in submissions to the Advisory Committee on Releases to the Environment*, available at http://www.defra.gov.uk/environment/acre/molecdata/pdf/acre_mdr_guidance.pdf

sequence. For each primer pair, detection of the target at or near the limit of detection should be demonstrated empirically by adding known concentrations of the target sequence to samples of the test DNA. These controls also serve to establish that components of the DNA sample do not prevent detection of the target sequence.

- 3.12 As well as reporting the experimentally determined detection limit of the particular tests, the certainty of the test result should also be considered. Delivering 100% certainty that a particular sequence is below the limit of detection usually requires the whole seed batch to be analysed – whenever a sub-sample only is analysed the certainty is less than 100%. Further details of the effect of sampling are provided in Annex 2.
- 3.13 In addition to expecting test results from consent holders and auditing the procedures used to produce that data, an additional measure would be a degree of independent testing by the GM Inspectorate. The aim of this testing should be to confirm the results provided by consent holders. This should follow a similar procedure outlined above for consent holder testing, and could be applied to all or a proportion of relevant consents.
- 3.14 In summary, the Committee **recommends** that, where seeds for Part B releases are not produced in contained facilities then additional requirements should be placed on consent holders to present data confirming the purity of the seeds to be used in the release and to provide the GM Inspectorate with a seed sample. The tests carried out by the consent holder should be independently confirmed by the GM Inspectorate in all or a proportion of cases.
- 3.15 As discussed above, and in Annex 2, it is not possible to confirm the complete absence of additional GM material from seed, so that some arbitrary threshold needs to be established. From the perspective of risk, and in practical terms a threshold of 0.1% presence by weight of seed delivered with a certainty of 95% is reasonable.
- 3.16 The Committee **recommends** that material for Part B releases should be considered to be free of additional GM material if it can be shown that such material is present in less than 0.1% (by weight) of the seed with a 95% certainty.

4. Monitoring during and following a release

- 4.1 Consents to release GM plants generally impose conditions that are required to manage potential risks, and it is the responsibility of the consent holder to ensure that any required risk management measures are implemented appropriately. In addition, the consent holder should monitor for unexpected consequences of the release, and act appropriately to manage any unanticipated risks. These requirements are important because the risk assessment is based on the assumption that risk management measures will be complied with, and it is also necessary to ensure that unexpected effects are taken into account. Both of these requirements will often extend beyond the period of release.
- 4.2 The GM Inspectorates check compliance with these objectives, through site visits both during the period of the release and throughout any subsequent management period. It must be stressed that the purpose of these visits is *not* to engage in risk management or monitoring *per se*, but to ensure that the consent holder is discharging their responsibilities in this regard.
- 4.3 A central issue concerning site visits for the purpose of inspection and enforcement is the frequency of those visits. Current practices by the GM Inspectorates involve an inspection frequency of 100% of sites visited at least once per annum. Clearly there are a number of possible regimes ranging from the permanent presence of an inspector on site throughout the trial and post-trial management period to no visits at all. Some of the factors determining visit frequency are outside the remit of the Committee (e.g. resource availability), but it is possible to offer some general guidance.
- 4.4 Considering the guiding principles described in paragraph 1.9, it is the case that only crops deemed to pose no additional risk will be released, and in reaching this conclusion the effect of a breakdown in risk management is generally considered. For example, while separation distances and other measures are often required to limit cross pollination between GM and non-GM crops, release would be unlikely to be recommended if the consequences of more extensive cross pollination, should it occur, were adverse. A second guiding principle is that inspection and enforcement should be proportionate to risk. Taken together these principles suggest that a small number of site visits for the purposes of inspection and enforcement is appropriate and that this number should be determined on a case-by-case basis.
- 4.5 The Committee **recommends** that site visits should continue to be part of the inspection and enforcement regime at the present rate and that ACRE should advise, as part of its routine casework, whether specific releases, or the risk management practices associated with them, require a higher frequency of site visits. It may also be appropriate for the Committee to give some general advice, on a case-by-case basis, of the timing of inspection visits.
- 4.6 In their submission to the sub group the Inspectorates requested ACRE's help in clarifying a number of more specific issues which are dealt with in the following paragraphs.

Do consents need to specify sowing rates?

- 4.7 Some applications for release specify both the number of plants to be released and the rate at which seed will be sown to achieve that number of plants. This sometimes presents problems for the consent holders because, due to variations in seed weight and

viability between batches, the specified plant numbers cannot be achieved using the specified sowing rate.

- 4.8 From the perspective of carrying out the risk assessment it is the number of plants released and the area into which they are released that is central. The sowing rate *per se* does not need to be taken into account, although it may be helpful if applicants provide an indication of the sowing rates that are expected to deliver the appropriate plant density¹¹.
- 4.9 The Committee therefore **recommends** that consents for release should continue to specify the area over which the release will take place, and the approximate number of plants to be released, and may give an indication of a range of appropriate sowing rates.

What constitutes ‘an effective pollen trap’?

- 4.10 A number of current consents refer to an optional pollen barrier for oilseed rape in order to limit cross-pollination. The Inspectorate have interpreted this as follows: ‘a pollen barrier should be specifically planted on all sides of a trial in order to physically trap pollen in the pollen barrier from all GM varieties that may be planted in the trial. Using part of the surrounding crops and/or any discard plots does not meet this function effectively.’
- 4.11 The Committee is content with the Inspectorate’s interpretation of ‘an effective pollen trap’.
- 4.12 The Committee **recommends** that pollen traps should continue to be used as a risk management measure. Pollen traps should be specifically planted for the purpose, and the nature of any pollen trap should be included in consent applications so that it can be assessed on a case-by-case basis.

What is the definition of ‘food chain’?

- 4.13 A number of consents specify that harvested material from the crops concerned should not enter the human food or animal feed chains, but do not specify whether grazing of the crop by animals during growth is permitted. It is also not clear whether grazing by wild animals is covered or whether the presence of trace amounts of GM pollen in honey contravenes this condition.
- 4.14 As part of its risk assessment ACRE considers the consequences of ingestion of the crop concerned by humans and animals. Release is only recommended if the available evidence suggests that the crop is not harmful to humans or animals, so that conditions requiring that material does not enter the food chain are purely precautionary. These precautionary measures should also apply to farm animals grazing the crop. However, the complete prevention of wild animals from grazing GM crops is not necessary, since if the crop were harmful it would not be released.
- 4.15 Considering pollen presence in honey, the effects of ingesting pollen in general are taken into account during risk assessment, and plants producing harmful pollen would not be recommended for release. Honey contains only trace amounts of pollen, and so this route only constitutes a negligible route of entry into the food chain.
- 4.16 The Committee **recommends** that the definition of ‘food chain’ used in consents should be made more specific and should specifically prohibit the grazing of farm animals on

¹¹ For example, consent holders could specify a median sowing rate with an indication of the variance required to account for the likely variation in germination rates.

GM crops unless feeding the crop to farm animals is a specific objective of the work for which consent is sought. Measures to exclude farm animals from the trial site should be specified in the consent.

What is the definition of 'neighbourhood'?

- 4.17 Several current consents refer to control of volunteers in the 'neighbourhood of release'. It is not clear whether this refers to the area where the GM crop is grown, this area together with any separation distances or a wider area surrounding the release site.
- 4.18 The purpose of volunteer control is to prevent cross pollination of GM plants with conventional plants in years following the GM release. Given that GM plants are released only into a specific area, and that cross pollination is only above specified limits within the separation zone, then volunteers only need to be controlled within the area encompassing the site where the GM plants are grown and the separation zones. Clearly the use of vague terms such as neighbourhood is unhelpful and should be avoided.
- 4.19 The Committee **recommends** that in future consents terms such as 'neighbourhood' should be avoided and replaced by precise specifications of over what areas risk management measures are required.

How should stubble from harvested GM release sites be treated?

- 4.20 Following the regrowth of oilseed rape stubble at some FSE sites in the winter of 2001 ACRE issued advice suggesting that consent holders should monitor sites for stubble regrowth, and take steps to avoid flowering of regrowth.
- 4.21 From the perspective of risk management the key issue is that flowering of stubble regrowth is prevented as a risk management measure – the regrowth *per se* does not pose a risk. As a result stubble regrowth should be controlled, either by cultivation or herbicide treatment, prior to flowering. The precise timing will depend on local conditions, and should be left to the discretion of the consent holder.
- 4.22 The Committee **recommends** that in future consents there should be an explicit requirement to control stubble regrowth, should it occur, before flowering, preferably by cultivation. Consents should not specify precise timings or control methods.

Should set aside be used as a follow crop after GM releases?

- 4.23 Generally consents specify what may not be grown in rotations following a GM release, largely with the objective of enabling adequate volunteer control for the purpose of restricting gene flow. This leaves open the possibility of prolonged set aside as a follow on crop.
- 4.24 Use of set aside as a follow on crop raises a number of issues for risk management. In particular precise adherence to set aside practices may compromise the control of volunteers and/or weeds. This is not to say that the practice is undesirable. Indeed from an environmental benefit perspective there are many benefits to the use of set aside which need to be taken into consideration. This need to be set against the central feature of the regulatory system – namely, that only crops deemed to pose no additional risk will be released. Clearly the use of set aside as a follow on crop needs to be evaluated as part of the original risk assessment of the release.
- 4.25 The Committee **recommends** that set aside should be permitted as a follow on crop after a GM release provided that any risks associated with the practice are adequately

and explicitly addressed in the environmental risk assessment that accompanies the application for consent to release. Additional risk management measures, particularly for volunteer control, may be required.

When and how should volunteers be controlled after harvest at GM release sites?

- 4.26 Control of volunteers before flowering is an important aspect of risk management at GM release sites. Its objective is to limit gene flow into non-GM varieties of the same crop and into sexually compatible weed species. However, it is important to see this management as primarily a precautionary measure, since the risk assessment prior to release consent being granted ensures that a GM crop that poses a risk would not be granted consent.
- 4.27 In the context of volunteer control, the scale of Part B releases needs to be borne in mind. For the vast majority of Part B releases volunteer control based on removal by hand is possible and desirable. Given that large scale releases of GM crops under Part B consents will be infrequent, the Committee expects volunteer control to be a straightforward and easily achieved risk management measure.
- 4.28 The Committee **recommends** that control of volunteers before flowering following GM crop releases should continue to be a condition of Part B releases consents. For small-scale releases it is reasonable to expect total volunteer control. Should applications be made for larger scale releases the environmental risk assessment should address the possibility that volunteer control will not be 100% effective, and the consent should reflect this, specifying volunteer control practices that are to be employed.

When and how should weeds be controlled at GM release sites?

- 4.29 Many consents specify that ‘related weeds’ need to be controlled, in the post-harvest period. The purpose of this condition is to limit gene flow between the crop and sexually compatible wild species.
- 4.30 The term ‘related weeds’ lacks precision and is open to a range of interpretations. This type of terminology is unhelpful since, at least to some extent, all plant species are related. It is necessary to be specific about which species are to be controlled, and to have clear criteria for the selection of these species.
- 4.31 The Committee **recommends** that consents should list the weed species that need to be controlled, and that the species should be limited to those where there is a significant risk of hybrids forming under the conditions of the release.
- 4.32 The importance of weed control, from a risk management perspective, will vary on a case-by-case basis, depending on the trait conferred by the gene(s) presented in the GM crop. For example, while transfer of herbicide tolerance traits to wild species may alter their fitness in an agricultural environment (and thus create a potential agronomic problem), it is much less likely to alter their ability to survive in a natural habitat. In contrast, it is possible to envisage other traits (e.g. pest resistance) that may alter the fitness of wild species under natural conditions.
- 4.33 The ease with which sexually compatible weeds can be controlled during the release (as opposed to in the post harvest period) will also vary on a case-by-case basis. For example, control of sexually compatible weeds in a crop of oilseed rape will be impossible, because of the difficulty in distinguishing weeds from oilseed rape plants prior to flowering or seed set. In this case, effective weed control in the post-harvest

period will be required. The size of the release will also determine the extent to which weeds can be controlled during cultivation of the GM crop.

- 4.34 It is also the case that for risk management weeds only need to be prevented from flowering when there are flowering GM plants present. If weed species have been controlled during cultivation and volunteer control is carried out effectively, then it may not be necessary to prevent flowering of weeds in the post trial period.
- 4.35 The Committee **recommends** that applications to release GM crops should contain detailed plans for weed management, and that these should be assessed on a case-by-case basis. The aim of these plans should be to reduce to a minimum gene flow between the crop and sexually compatible weeds, and should, where possible, focus on preventing the flowering of weeds while the crop is flowering.

5. Summary of recommendations

- 5.1 Paragraph 3.5: The Committee would, therefore, **recommend** different inspection procedures depending on the conditions under which seed or other propagules are produced, and the nature of the propagule (seed vs. vegetative). A detailed description of the conditions under which seeds are to be produced should be included in applications for consent to release.
- 5.2 Paragraph 3.7: If seeds or other propagules are produced under conditions of genetic isolation or the propagules are vegetative then the Committee **recommends** that the present system of management audits should be maintained
- 5.3 Paragraph 3.14: The Committee **recommends** that, where seeds for Part B releases are not produced in contained facilities then additional requirements should be placed on consent holders to present data confirming the purity of the seeds to be used in the release and to provide the GM Inspectorate with a seed sample. The tests carried out by the consent holder should be independently confirmed by the GM Inspectorate in all or a proportion **of** cases.
- 5.4 Paragraph 3.16: The Committee **recommends** that material for Part B releases should be considered to be free of additional GM material if it can be shown that such material is present in less than 0.1% (by weight) of the seed with a 95% certainty.
- 5.5 Paragraph 4.5: The Committee **recommends** that site visits should continue to be part of the inspection and enforcement regime at the present rate and that ACRE should advise, as part of its routine casework, whether specific releases, or the risk management practices associated with them, require a higher frequency of site visits. It may also be appropriate for the Committee to give some general advice, on a case-by-case basis, of the timing of inspection visits.
- 5.6 Paragraph 4.9: The Committee therefore **recommends** that consents for release should continue to specify the area over which the release will take place, and the approximate number of plants to be released, and may give an indication of a range of appropriate sowing rates.
- 5.7 Paragraph 4.12: The Committee **recommends** that pollen traps should continue to be used as a risk management measure. Pollen traps should be specifically planted for the purpose, and the nature of any pollen trap should be included in consent applications so that it can be assessed on a case-by-case basis.
- 5.8 Paragraph 4.16: The Committee **recommends** that the definition of 'food chain' used in consents should be made more specific and should specifically prohibit the grazing of farm animals on GM crops unless feeding the crop to farm animals is a specific objective of the work for which consent is sought.
- 5.9 Paragraph 4.19: The Committee **recommends** that in future consents terms such as 'neighbourhood' should be avoided and replaced by precise specifications of over what areas risk management measures are required.
- 5.10 Paragraph 4.22: The Committee **recommends** that in future consents there should be an explicit requirement to control stubble regrowth, should it occur, before flowering, preferably by cultivation. Consents should not specify precise timings or control methods.

- 5.11 Paragraph 4.25: The Committee **recommends** that set aside should be permitted as a follow on crop after a GM release provided that any risks associated with the practice are adequately and explicitly addressed in the environmental risk assessment that accompanies the application for consent to release. Additional risk management measures, particularly for volunteer control, may be required.
- 5.12 Paragraph 4.28: The Committee **recommends** that control of volunteers before flowering following GM crop releases should continue to be a condition of Part B releases consents. For small-scale releases it is reasonable to expect total volunteer control. Should applications be made for larger scale releases the environmental risk assessment should address the possibility that volunteer control will not be 100% effective, and the consent should reflect this, specifying volunteer control practices that are to **be** employed
- 5.13 Paragraph 4.31: The Committee **recommends** that consents should list the weed species that need to be controlled, and that the species should be limited to those where there is a significant risk of hybrids forming under the conditions of the release.
- 5.14 Paragraph 4.35: The Committee **recommends** that applications to release GM crops should contain detailed plans for weed management, and that these should be assessed on a case-by-case basis.

Terms of reference

- To consider the suitability and effectiveness of current consent conditions from the perspective of the Inspection and Enforcement regime. The review will examine recent cases for lessons that can be learned, and recommend potential improvements to consent conditions for the future.
- To consider what requirements should be placed on consent holders for the provision of information (including molecular data) and samples to support the Inspection and Enforcement regime.

List of members

ACRE members

Professor Alan Gray (Chair of sub group)

Professor Phil Mullineaux

Professor Chris Pollock

Mr Jim Orson

Members from outside ACRE

Mr Mark Ball (Food Standards Agency)

Dr Rosie Hails (Agriculture and Environment Biotechnology Commission)

Limitations on GMO detection in seeds using the polymerase chain reaction (PCR).

Theoretically, a single copy of the target sequence can be detected by the polymerase chain reaction (PCR), although, depending on the primers used and the size of the target sequence, this limit can be as high as 10 copies. The ability to detect a very small number of target copies gives a very small, but finite limit of detection (in terms of percentage presence of the target sequence) which varies from species to species. This is because the amount of DNA present in the genome of different species varies. For example a 100 ng sample of DNA¹ contains approximately 37,000 copies of the maize genome, while an equivalent amount of DNA from oilseed rape contains approximately 87,000 copies of the oilseed rape genome. The absolute limit of detection of the PCR test is the presence of one copy of the genome containing the transgene, which equates to 0.003% for maize and 0.001% for oilseed rape.

However, in practice it is not possible to detect GM presence at levels that are even close to the theoretical possibilities because of sampling error.

The following example illustrates the nature of sampling error. Consider withdrawing black and white marbles from a bag containing 50 black marbles and 50 white marbles. If 4 marbles are removed at random what is the chance of withdrawing 4 black marbles? The chance of the first marble being black is 50/100, for the second 49/99, for the third 48/98, and for the fourth 47/97. To calculate the overall probability the individual probabilities are multiplied together, so that 4 black marbles will be withdrawn around 6% of the time. This means that if you use the withdrawal of a sample of 4 marbles from the bag to test for the presence of white marbles, 6 tests in every 100 will give the incorrect answer. Clearly, the larger the sample the more certainty can be achieved. If a sample of 5 marbles is used only 3 tests in 100 gives the wrong answer, and with 6 only 1 test in 100. For this particular example to achieve 100% certainty 51 marbles need to be sampled. If the mixture contained 95 black and 5 white marbles, 96 would need to be sampled for 100% certainty, and a sample of 4 would give an incorrect result in 80 tests out of 100. Similarly if there were 99 black and 1 white all 100 marbles would need to be examined to be 100% certain, and a sample of 4 would give an incorrect result 96 times out of 100.

Sampling error considerations impose problems at a minimum of two stages during the analysis of GM presence in seeds.

1. During the sampling of seeds for analysis
2. During sub-sampling of DNA preparations for analysis

Testing samples for the presence of specific DNA sequences is destructive, so that only a sub sample of any batch of seed can be tested. As a result of this limitation it is not practical for any test to deliver 100% certainty that a specific sequence is not present above the limit of detection.² The following table summarises the relationship between sample size and the level of GM presence that can be detected with specific degrees of certainty.

¹ In practical terms this is the maximum amount of DNA that can be analysed in a single PCR test.

² Further details can be found in http://biotech.jrc.it/doc/EuroReport_sampling_strategies.pdf, which is also the source for the table below.

Sample size	Limit of GM detection (as %) with certainty of:		
	90%	95%	99%
100	2.28	2.95	4.50
200	1.14	1.49	2.28
300	0.76	0.99	1.52
400	0.57	0.75	1.14
800	0.29	0.37	0.57
1200	0.19	0.25	0.38
2000	0.12	0.15	0.23
2500	0.09	0.12	0.18
3000	0.08	0.10	0.15
6000	0.04	0.05	0.08
10000	0.02	0.03	0.05

From this table, it can be seen that a negative result from a test carried out on a sample of 3000 seeds means that there is a 95% certainty that the sequence tested for is not present at a level of 0.1% or higher.

The removal of samples from DNA preparations for analysis also introduces errors of a similar nature³. Taken together these sampling errors mean that detection of GM presence at levels below 0.1% is not practical.

³ Kay, S. and Van den Eede, G (2001) The limits of GMO detection. *Nature Biotechnology* **19**, 450